Locus-coeruleus norepinephrine system function in a developmental animal model of schizophrenia: the socially isolated rat

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-ABSTRACT-

Introduction

Schizophrenia is a chronic, debilitating mental disorder characterised by positive, negative and cognitive symptoms. Current treatment regimens fail to adequately address the cognitive and negative symptoms of the disorder. Social isolation rearing (SIR) is a well-established developmental adversity paradigm which is used as an animal model of schizophrenia and usually studied in male rats. Previous SIR studies have found attentional abnormalities in isolated rats in behavioural tests which correspond to the results of studies investigating the cognitive symptoms of schizophrenia in patient trials. Isolated rats also display abnormal social responses which may be of relevance to the negative symptoms of schizophrenia. The primary aim of this study was to build on existing SIR literature by performing behavioural tests in socially isolated rats to address attentional function. Neurochemical investigations were performed on projections of the locus coeruleus norepinephrine system, known to be involved in attentional function, as research on this system is surprisingly sparse. The secondary aim of the study was to address the negative symptoms of schizophrenia using ultrasonic vocalisation recording to investigate the calling behaviour of isolated rats in response to a novel context. The study included both male and female rats so that sex differences could be studied in the context of social isolation.

Methodology

Sprague-Dawley rats were weaned at postnatal day (p) 21 and randomly allocated to one of four housing groups; female socialised (n=50), female isolated (n=50), male socialised (n=38) and male isolated (n=38). Socialised animals were housed 4 per cage (single sex) and isolated animals were housed alone. Animals were weighed and cages cleaned weekly as part of a minimal handling protocol required for SIR. After 8 weeks in their housing conditions (p78-82) rats underwent one of two behavioural paradigms: three phase novel object recognition or ultrasonic vocalisation recordings. Between p90-94 animals were rapidly decapitated and the hippocampus and prefrontal cortex were dissected out for use in one of two neurochemical analyses. For *in-vitro* superfusion experiments the tissue was used immediately to quantify functional release of radioactively-labelled norepinephrine when stimulated with glutamate under varying conditions. Enzyme linked immunosorbent assays (ELISA) and bicinchoninic acid (BCA) protein assays was performed to quantify norepinephrine and glutamate concentrations expressed in relation to the wet weight of the tissue and amount of protein in the tissue.

Results

Behavioural and neurochemical changes were induced by the SIR model. Isolated animals were found to respond to novel objects abnormally compared to control animals. During initial exposure to a novel environment in the first phase of the novel object recognition test isolated animals demonstrated hypoactivity. An overall reduction in the fractional release of norepinephrine when stimulated with combinations of glutamate and gamma-aminobutyric acid (GABA) was demonstrated in the hippocampus of isolated rats. Sex differences were evident in a number of experiments. Female rats were found to be hyperactive in the three phases of the novel object recognition test compared to males and also had elevated hippocampal norepinephrine activity as well as an increased concentration of norepinephrine in this area. Male rats on the other hand had an elevated prefrontal cortex norepinephrine activity and concentration.

Conclusion

The SIR paradigm is able to induce behavioural and neurochemical changes in both female and male rats. The results of this study reinforce the usefulness of SIR as a model for schizophrenia as the way in which isolated animals responded to novel objects was different to their socialised counterparts. This difference implies an abnormal attentional response which corresponds to the cognitive symptoms described in schizophrenia. Furthermore, the neurochemical experiments performed in this study are the first of their kind and provide preliminary evidence for the GABAergic mechanisms underlying attentional abnormalities associated with SIR. The prevalence of sex differences throughout testing also provides strong evidence for the inclusion of both sexes in future studies to avoid the omission of potentially important findings. Future studies to refine and build on neurochemical analyses in developmental models of schizophrenia, such as SIR will potentially provide a mechanistic understanding of cognitive dysfunction as well as useful translational information for treating the human disorder.

-DECLARATION-

I, Katherine Helen Atmore, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university. I empower the University of Cape Town to reproduce, for the purpose of research, either the whole or any portion of the contents of this dissertation in any manner whatsoever.

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TABLE OF CONTENTS

-LIST OF ABBREVIATIONS-

[^{3}H]DA- Tritiated dopamine

[^{3}H]NE- Tritiated norepinephrine

AEC- Animal ethics committee

AMPAR- α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (glutamate) receptor

ANOVA- Analysis of variance

Ant- Antagonist

BCA- Bicinchoninic acid

$CaCl_2.H_2O$- calcium chloride dihydrate

CNQX- competitive AMPA/kainate receptor antagonist

DA- Dopamine

DPM- Decays per minute

EDTA- ethylenediminetera-acetic acid disodium salt

ELISA- Enzyme linked immunosorbent assay

FI- Female isolated

FS- Female socialised

GABA- Gamma (γ)-aminobutyric acid

Glu- Glutamate

HC- Hippocampus

HPLC- High performance liquid chromatography

HSF- Health science faculty

IPO- In the presence of

IQR- Interquartile range

Iso- Isolated

IZ- Inner-zone

KCl- Potassium chloride

K^+ - Potassium

Max- Maximum

$MgCl_2.6H_2O$- Magnesium chloride hexahydrate

MI- Male isolated

Min- Minimum

Min(s) - Minute(s)

MK-801- Uncompetitive antagonist of the NMDA receptor

MRI- Magnetic resonance imaging

MRS- Magnetic resonance spectroscopy

MS- Male socialised

n- Animal number

NaCl- Sodium chloride

$NaH_2PO_4.H_2O$- Sodium dihydrogen phosphate monohydrate

$NaHCO_3$- Sodium hydrogen carbonate

NaOH- Sodium hydroxide

NE- Norepinephrine

NMDAR- N-Methyl-D-aspartate (glutamate) receptor

NOR- Novel object recognition

O- Object

p- Post-natal day

p- Probability value

P. - Phase

PET- Positron emission tomography

PFC- Prefrontal cortex

Q- Quadrant

RIPA- Radio immunoprecipitation assay

RPM- revolutions per minute

RT-PCR- Reverse transcription polymerase chain reaction

S-D- Sprague-Dawley

SD- Standard deviations

SF- Superfusion

SIR- Social isolation rearing

Soc- Socialised

UCT- University of Cape Town

USV- Ultrasonic vocalisation

vs. – Versus

-LIST OF FIGURES-

-LIST OF EQUATIONS-

-LIST OF TABLES-

CHAPTER 1
-INTRODUCTION-

1.1 BACKGROUND ON SCHIZOPHRENIA

1.1.1 DEFINITION, SYMPTOMS AND DIAGNOSIS

Schizophrenia is a highly complex and debilitating neuropsychiatric disorder which typically persists chronically once an initial diagnosis is made. The disorder is cyclical and characterised by psychotic episodes interspersed with periods of remission and accompanied by a progressive deterioration in mental function (Millier, Schmidt et al. 2014). Psychosis refers to an impairment of thought so severe that contact is lost with reality (Gaebel and Zielasek 2015). Formalised definitions of schizophrenia can be found in diagnostic manuals DSM-V (American Psychiatric Association 2013) and ICD-10 (World Health Organization 1992), the criterion for diagnosis specified in these manuals are subject to ongoing update and review (Biedermann and Fleischhacker 2016). Diagnoses are based on the patient's reported experience as well as the observations of the practitioner. The symptoms of schizophrenia fall in three broadly defined categories.

The positive symptoms of schizophrenia are so-called as they are additive to normal experience. Delusions, hallucinations (usually auditory) and disorganised thoughts, speech and movements are the most typical positive symptoms. These symptoms contribute to what is known as 'active phase' schizophrenia or psychosis where the patient experiences a severe disconnection with reality. Often the delusions and hallucinations are persecutory or paranoia inducing (Mueser, Bellack et al. 1990). This active-phase is what the public typically associate with schizophrenia, however positive symptoms are rarely experienced continuously and tend to fluctuate in severity (Erritty and Wydell 2013). In reality it is the negative and cognitive symptoms which make up the majority of the individual's experience of the disorder meaning that these other symptoms are the most debilitating (Carbon and Correll 2014). Positive symptoms are largely understood to be mediated by dysfunction of the dopamine (DA) system. Antipsychotic medications are used to manage positive symptoms and reduce active-phases of the disorder (Bruijnzeel, Suryadevara et al. 2014).

The negative symptoms of schizophrenia are deficits in function and include apathy (emotional blunting) evidenced in body language, facial expression and tone of voice, avolition (loss of motivation), anhedonia (inability to experience pleasure), alogia (poverty of speech) and societal withdrawal (An der Heiden, Leber et al. 2016). Decreased social function can be apparent before a diagnosis of the disorder and often becomes increasingly problematic and debilitating with time (Hansen, Torgalsbøen et al. 2009). Negative symptoms are often apparent before a diagnosis is made. They are known as the residual or prodromal phase of schizophrenia as they persist even when positive symptoms are not present (Gourzis, Katrivanou et al. 2002). This indicates that negative symptoms have their own distinct underlying mechanism. This theory is strengthened by the fact that most pharmacological interventions for schizophrenia do little to address the negative symptoms (Tsapakis, Dimopoulou et al. 2015).

The cognitive symptoms of schizophrenia have a detrimental impact on numerous higher functions. At present, an increased research focus is being directed towards these cognitive symptoms in order to provide a more complete understanding of schizophrenia. Initiatives such as MATRICS – (Measurement and Treatment Research to Improve Cognition in Schizophrenia) (Marder and Fenton 2004, Lustig, Kozak et al. 2013) have attempted to systematically address these symptoms and provide novel therapeutic targets for their relief (Lin, Tsai et al. 2014). Cognitive symptoms include the weakening of declarative and working memory faculties. The attentional system is also often found to be dysregulated in sustained attention and attention orienting tasks (Shen, Popescu et al. 2014). This implies an overarching abnormality in salience processing (Andersen, Campbell et al. 2016). An overall decrease in processing speed is also typically experienced, this may stem from a dependence on non-typical neural pathways of executive function (Knowles, Weiser et al. 2015). These deficits contribute to difficulties in absorbing and interpreting information and the enacting of an appropriate response (Heinrichs and Zakzanis 1998). This means that day to day activities and engagement with society becomes increasing difficult even if a medication regime is adhered to (Marder 2006).

The DSM-V details five core symptoms for diagnostic purposes, these being delusions, hallucinations, disorganised thoughts and speech, abnormal motor behaviour and negative symptoms. A diagnosis of schizophrenia requires the presence of at least two of the five core symptoms in one month, with one of these being delusions, hallucinations or disorganised speech. In addition to this, evidence of decreased life skills and other disturbances and symptoms over a six month period are required to rule out acute psychoses. Features of anxiety and depression are also often concurrent with schizophrenia. This wide array of clinical features of the disorder means that

schizophrenia is notoriously heterogeneous which can complicate diagnosis (Tandon, Gaebel et al. 2013). There may also be difficulty in differentiating between schizophrenia and bipolar in certain cases (van Os and Kapur 2009).

1.1.2 PREVALENCE AND AETIOLOGY

Schizophrenia is commonly reported in the literature as affecting 1% of the global population (Chen, Cao et al. 2015). Recent reviews have made attempts to demystify this value and found that the prevalence is likely to be slightly lower than 1% depending on the criterion of inclusion (McGrath, Saha et al. 2008, Simeone, Ward et al. 2015). The disorder forms a huge burden to the individual, their families, society as a whole (Millier, Schmidt et al. 2014), and the economy (de Silva, Hanwella et al. 2012). This is due to the disabling features of the disorder meaning that one's ability to lead an independent life is severely compromised. The ratio of male to female incidence is approximately 1.4:1, yet prevalence between the sexes is roughly equal (Aleman, Kahn et al. 2003), this disparity has been attributed to the worsened life expectancy of males with the disorder (Leung and Chue 2000). The diagnosis of schizophrenia typically occurs during late adolescence or early adulthood, notably diagnosis in females is often slightly later than in males (Häfner, an der Heiden et al. 1998). Schizophrenia does not 'begin' at this age, however this is the time at which the positive symptoms of the disorder most often reach the threshold required for a clinical diagnosis, having persisted for at least 6 months. The time prior to a diagnosis is known as the prodromal phase of the disorder and negative and cognitive symptoms are often already present at this stage (Fisher, Loewy et al. 2013).

Schizophrenia is notoriously elusive in its origins, and once a diagnosis of the disorder has been made there are many other factors which add to its complexity. Assessing and improving the quality of life of individuals represents a major challenge to health professionals (Awad and Voruganti 2012). The World Health Organisation listed schizophrenia in the top 6 of the leading worldwide causes of global disease burden (World Health Organisation 2008). Societal stigmatisation of individuals experiencing schizophrenic symptoms is common and due to a lack of understanding of the disorder and the misconception that schizophrenia makes people violent and a threat to the public. The truth is that the risk posed to the individuals themselves is far greater. Suicide is rife in schizophrenic patients with 2-10% choosing to end their own lives (Gómez-

Durán, Martin-Fumadó et al. 2012, López-Moríñigo, Ramos-Ríos et al. 2012) and up to 50% making attempts (Bolton, Gooding et al. 2007). Other associated issues are unemployment, homelessness, poor diet, drug and alcohol abuse, thus the life expectancy of those with a diagnosis of schizophrenia has been found to be reduced by 12-15 years (McGrath, Saha et al. 2008, van Os and Kapur 2009). These issues are often be exacerbated by the progressive social withdrawal of the individual contributing to feelings of loneliness and hopelessness (Shrivastava, Bureau et al. 2013).

In South Africa, the administration of effective treatment is particularly challenging, especially in 'at risk' individuals of lower socio-economic status bracket (Lund and Flisher 2002). There are many people living with a diagnosis of schizophrenia and many more who are undiagnosed. Access to medication and support is often limited and may be accompanied by low levels of education which translate to poor health-related practices. Further complications are the fact that people living with HIV/AIDS are more likely to be develop disorders such as schizophrenia but health professionals are not as well equipped to identify and deal with the latter (Mall, Sorsdahl et al. 2012). There are differences in understanding of the disorder amongst different cultural groups, witchcraft and possession by spirits are believed by some to be the cause of the disorder (Mbanga, Niehaus et al. 2002) and stigmatisation by communities is common (de Wet, Swartz et al. 2015). In these instances individuals are less likely to seek treatment and adhere to their medications, which results in high psychotic relapse. This is especially the case in South African populations where methamphetamine abuse and hence, methamphetamine-induced psychosis is common (Akindipe, Wilson et al. 2014), these individuals are highly difficult to treat due to drug seeking behaviour occluding their desire to be treated. This increases the number of those living untreated with schizophrenia and other psychotic disorders. Limited access to health care services and associated costs are also a preventative factor for patients. Time spent waiting in clinics as well as distances needed to be travelled in order to reach clinics push the perceived benefit versus cost ratio much towards the cost side. Lack of funding for specialist training of health care professionals and other communities members is also problematic (Lund and Flisher 2002), particularly when language barriers are taken into consideration (Asmal, Mall et al. 2011). This research has the potential to increase our understanding of schizophrenia and in doing so will allow for more effective education of the abovementioned 'at risk' individuals in this country as well as associated carers.

The causative agents of schizophrenia are largely unknown, both genetic and environmental factors have been investigated and implicated. A meta-analysis of data from twin studies found that genetic similarity does increase the likelihood for a diagnosis (heritability estimates are around 80%) and reinforced the idea the schizophrenia is complex and multifactorial disorder (Sullivan, Kendler et

al. 2003). In recent years, genome wide association studies have shed light on a large number candidate genes which in combination may contribution to the development of the disorder (Chen, Cao et al. 2015). As with many psychiatric disorders, in the absence of a clear-cut genetic contribution, research focus has moved towards studying other factors which bring about the disorder in those with a genetic predisposition. Epigenetic processes are receiving increasing focus to elucidate the mechanisms underlying gene expression and regulation and what part this may have to play in the disorder (Dempster, Viana et al. 2013). Environmental risk factor studies have revealed that the prevalence of schizophrenia is increased in urbanised individuals (Mortensen, Pedersen et al. 1999), immigrants (Eaton and Harrison 2000) and those living in countries high levels of income inequality (Burns, Tomita et al. 2014). What may link these commonly reported risk factor is the likelihood for social exclusion (Morgan, Kirkbride et al. 2008). The conditions of birth and pregnancy are also thought to play a role in the development of the disorder (Cannon, Jones et al. 2002). The comorbidity of substance abuse disorders and schizophrenia is relatively high, around 50% (Addington and Addington 2007). A common perception of the public is that the use of illicit substances (particularly cannabis) can cause schizophrenia. While it is certainly true that some drugs can induce effects that mimic the disorder in the short term, it is difficult to prove a longer-lasting effect (Murray, Paparelli et al. 2013). The interpretation of studies investigating this relationship is highly complex. The age at which drug abuse typically begins often coincides with the prodromal phase of the disorder (Henquet, Krabbendam et al. 2005). Possible explanations for this relationship are that drug-taking is a form of self-medication for cognitive symptoms prior to a diagnosis or that substance abuse can induce psychosis in genetically-predisposed individuals which precipitates a diagnosis (Bizzarri, Rucci et al. 2009, Sara, Burgess et al. 2014). Studies to identify gene-environment interactions are being conducted to integrate increasingly large data sets. Though there are many methodological challenges which needed to be accounted for, these collaborative efforts may shed new light on the complexities of schizophrenia's aetiology (van Os, Rutten et al. 2014).

1.1.3 TREATMENT AND NEUROCHEMICAL HYPOTHESES

Though schizophrenia is most often intractable, it can be managed with pharmacological and psycho-social interventions. Pharmacological interventions for schizophrenia target the positive symptoms of the disorder and are known as the antipsychotics. Antipsychotic medication falls into

two broad categories, ‘typical’ (first generation) antipsychotics and ‘atypical’ (second generation). Typical antipsychotics antagonise the dopaminergic system D_2 receptors in the mesolimbic area of the brain (Carlsson 1978). These drugs may be very effective in reducing length, frequency and severity of psychotic episodes, so much so that after their serendipitous discovery in the 1950s they were hailed as a miracle cure. However, extra-pyramidal side-effects of the motor system are common from the dopaminergic antagonism in the mesolimbic area (Tandon 2007). The newer atypical antipsychotics act on the dopaminergic system, though with reduced affinity for D_2 receptors, as well as antagonising the serotonergic system 5-HT_{2A} receptors. These drugs do not induce extra-pyramidal side-effect to the same extent as their predecessors (Dazzan, Morgan et al. 2005). Despite the reduction of side-effects atypical medications have been found to be only ‘inconsistently more effective’ in addressing negative symptoms and cognitive symptoms and are also considerably more costly than first generation alternatives (Tandon, Belmaker et al. 2008). It is also known that up to 30% of individuals are treatment-resistant, given that they continue to experience positive symptoms even after adhering to antipsychotic regimes (Hasan, Falkai et al. 2012). The existence of this subgroup strongly suggests that there is more than just DA dysfunction underlying psychotic states and demonstrates a crucial need for increased mechanistic understanding of the disorder. Aside from those who are treatment-resistant there are many more individuals who are undiagnosed, do not have access to, or are not prescribed appropriate treatment (Kennedy, Altar et al. 2014). Another factor which complicates the treatment of schizophrenia is patient compliance. Given the profile of symptoms, particularly cognitive memory deficits, it is difficult to ensure patients remain on course with their medication. It has been found that 74% of patients stop taking their medication within 18 months (Lee, Kane et al. 2011). Nowadays, in developed countries, pharmacological treatments are most often given in tandem with psycho-social therapies. There are a diverse range of treatments available, from community counselling groups and family therapy through to art and yoga therapies (Cormac, Jones et al. 2002, Asmal, Mall et al. 2011, Crawford, Killaspy et al. 2012, Cramer, Lauche et al. 2013). Effectiveness of these therapies is highly variable, though they have been shown to facilitate adjustment to living with residual symptoms and reintegrating into society once active phase symptoms have subsided.

Given its heterogeneity there is no single neurotransmitter system which can account for all the symptoms of schizophrenia. The effects of different drug types on the profile of symptoms of schizophrenia have however led to the development of various neurochemical hypotheses. The ‘dopamine hypothesis’ of schizophrenia is well established given the efficacy of DA antagonism as an antipsychotic treatment. DA is a monoamine neurotransmitter which acts on G-protein coupled

receptors (D1- D5) (Beaulieu, Espinoza et al. 2015). It states that hyperactivity at the D_2 receptor, especially in the mesolimbic region of the brain gives rise to psychotic aspects of the disorder (Carlsson 1977, Iversen and Iversen 2007). This theory is corroborated by the fact that the there is a positive correlation between the affinity of a drug for the D_2 receptor and its clinical efficacy (Seeman, Chau-Wong et al. 1975, Madras 2013). Whilst DA antagonists serve to ameliorate positive symptoms, DA agonists are known to cause induction of psychotic states in both affected and non-affected individuals (Angrist, Sathananthan et al. 1974, Featherstone, Kapur et al. 2007). It is theorised that sensitisation of the dopaminergic system may underlie the induction of psychotic states in the disorder (Seeman, Weinshenker et al. 2005). The evidence underlying the dopamine hypothesis of schizophrenia's positive symptoms is compelling whereas the mechanisms behind the negative and cognitive symptoms are less clear cut. It is suggested that dopaminergic hyperactivity in the mesolimbic system is accompanied by dopaminergic hypofunction in the mesocortical region. This 'hypofrontality' thought to account for some of the negative and cognitive symptoms of schizophrenia and evidenced by reduction function of the frontal cortex (Andreasen, O'Leary et al. 1997) as evidenced in imaging studies during mental tasks (Callicott, Mattay et al. 2003).

Glutamate is the major excitatory neurotransmitter in the brain and acts on ionotropic receptors (AMPA, NMDA and kainate) as well as on metabotropic glutamate receptors (mGluRs 1 - 8) (Niswender and Conn 2010). More recently research has moved towards glutamate hypotheses of schizophrenia given the ability of dissociative anaesthetics phencyclidine (PCP) and ketamine to induce states that are clinically indistinguishable from the disorder in unaffected individuals, as well as exacerbating symptoms in affected individuals (Javitt and Zukin 1991, Newcomer, Farber et al. 1999). PCP and ketamine are both ionotropic glutamate NMDA receptor antagonists. It is thought that decreased functioning of NMDA receptors also contributes to hypofrontality in schizophrenia. Despite these findings, the glutamate system remains untargeted for treatment given that it is the major excitatory neurotransmitter in the body. The pharmacological upregulation of the glutamate system by the direct stimulation of NMDA receptors would likely lead to the induction of seizures and neurotoxicity. For this reason attempts have been made to upregulate NMDA function by bypassing glutamate and increasing the availability of co-agonists, such as glycine (Möhler, Boison et al. 2011) and D-serine (Heresco-Levy, Javitt et al. 2005). This line of enquiry has been strengthened by findings that levels of D-serine in patients with schizophrenia are reduced (Hashimoto, Engberg et al. 2005). Where there is a glutamate dysfunction an accompanying disturbance in GABA function is highly likely given the synergistic relationship of excitation and inhibition between the two. GABA neurons often serve as interneurons in brain for example in the

hippocampus and regulate glutamatergic transmission via feedback inhibition (Andreasen and Lambert 1991), this reciprocal relationship may be impacted in schizophrenia. Recent studies have attempted to address this relationship and a number of studies have found genetic GABA alterations in schizophrenia (Schubert, Föcking et al. 2015) as well as in *in-vivo* imaging studies (Frankle, Cho et al. 2015).

Literature on the dopamine and glutamate hypotheses is abundant and investigation into other neurotransmitters is required to provide a fuller picture of the multisystem dysfunction which is likely to bring about schizophrenia. Given the importance of dopamine function in schizophrenia this warrants the study of other monoaminergic neurotransmitters. Norepinephrine (NE) is synthesized directly from dopamine and is thought to play role in many cognitive functions; attention, memory, arousal and behavioural response to stress (Berridge and Waterhouse 2003). All of these processes are in some way or another implicated in schizophrenia and yet to be targeted successfully for treatment. NE binds to α_1 and α_2 as well as β_1, β_2 and β_3-adrenoceptors (Haggerty, Glykos et al. 2013). All adrenoceptors can be found post-synaptically with α_2 receptors also localising to the presynaptic terminal of noradrenergic neurons where they serve as autoreceptors regulating negative feedback (Berridge and Waterhouse 2003). NE release can be triggered by the excitatory action of glutamate on AMPA and NMDA receptors on presynaptic NE neurons (Howells and Russell 2008). Glutamate release may also be triggered by adrenoceptor activation (Chen, Li et al. 2006). The reciprocal relationship between these two neurotransmitter systems may be altered in a schizophrenic context. The elevated NE hypothesis of schizophrenia is a relative newcomer in the literature. Previous studies on NE levels in cerebrospinal fluid and blood have revealed increased concentrations associated with schizophrenia as well as positive correlations to psychotic symptoms (Kemali, Del Vecchio et al. 1982, Dajas, Barbeito et al. 1983). Attempts to link the function of the monoamine neurotransmitters have posited that in the frontal cortex dopamine is transported by NE uptake transporters due to relatively low concentrations of dopamine transporters (Morón, Brockington et al. 2002). This work is predominately framed in the context of attention deficit hyperactivity disorder but may well have relevance to schizophrenia. Additionally in certain cases dopamine can activate α and β-receptors (Yang, Zhang et al. 2014) given the strong evidence for dopamine dysfunction in schizophrenia it follows that the closely related NE system may also be impacted. Recent work has investigated the therapeutic effect of targeting the NE system in patient trials. Reboxetine, a NE reuptake inhibitor which increases the availability of NE at the synapse, is being trialled to quantify amelioration of cognitive function. Reboxetine seems to be well tolerated but has not been able to consistently produce benefit in

schizophrenia (Poyurovsky, Faragian et al. 2009, Bruno, Zoccali et al. 2014). Given its posited role in schizophrenia, studies have been conducted to explore whether there are differences in the synthesis of NE. The majority of NE is made in a small nucleus of the brain; the locus-coeruleus (LC) (Grzanna and Fritschy 1991). Investigations into the anatomical abnormalities of the noradrenergic LC tissue post mortem found no differences when staining for tyrosine hydroxylase, the rate limiting enzyme responsible for the conversion of dopamine to NE, between samples from schizophrenia patients and controls (Craven, Priddle et al. 2005). In another post mortem study, cell number and volume were quantified revealing an increase in the volume of LC neurons in schizophrenia patients but no differences in cell number (Marner, Søborg et al. 2005).

As previously stated, one of the cognitive symptoms of schizophrenia is attentional dysfunction. In the healthy brain attention processing is facilitated by activation of the LC-NE system (Clark, Geffen et al. 1987, Schwarz and Luo 2015), this has been confirmed by work done in our lab (Howells, Stein et al. 2012). LC neurons fire in two different patterns; tonic firing which is regular discharge during wakefulness and phasic firing which increases on presentation of task relevant stimuli (Howells, Stein et al. 2012). Its activation by salience, stressors or novelty increases NE release in its multiple target regions. Its connections are well characterised, particularly to the prefrontal cortex and hippocampus (Moore and Bloom 1978). These areas are associated with attentional processing (Kõiv, Zobel et al. 2011) and pathological processes of schizophrenia have been described in both of these regions. It has been repeatedly demonstrated that hippocampal volume is decreased in post mortem tissue from schizophrenia patients and there is also evidence of decreased cell proliferation (Allen, Fung et al. 2016). As previously mentioned, there is also a wealth of data relating to prefrontal cortex dysfunction in schizophrenia, notably the hypofrontality hypothesis. Preliminary anatomical investigations revealed that bilateral lesioning of the LC noradrenergic bundles resulted in an inability to ignore task irrelevant stimuli (Mason and Lin 1980). This is of particular interest in the context of schizophrenia since deficits are commonly found in selective attention studies utilising distractors, for example the Stroop task (Mayer, Hanlon et al. 2015). Schizophrenia patients have also been shown to exhibit deficits in tests for other types of attentional processing. In orienting attention studies impairments in the visual tracking of stimuli has been described (Mather and Putchat 1984). More recently this has been visualised during *in-vivo* brain imaging studies as a reduction in the amplitude of an event related potential known as P3 or P300 which is concurrent with the presentation of salient stimuli in healthy individuals (Laurens, Kiehl et al. 2005). Deficits are also evident in attention switching studies of schizophrenia (Smid, Martens et al. 2013). These tests provide information not only on

attention faculties but also indicators of processing speed and working memory, it is impossible to fully separate these closely linked functions. Despite attentional dysfunction being particularly robust and well described finding in schizophrenia (Nuechterlein, Barch et al. 2004, Knowles, Weiser et al. 2015) surprisingly the LC NE system remains understudied in this context. Notably, many of the aforementioned studies were conducted in the 1980s and the LC-NE system is less well studies with modern techniques. The present study was designed with the intention of addressing this gap in the literature and attempting to draw together attentional dysfunction as well as underlying neurochemistry associated with to the LC NE system.

1.2 MODELLING SCHIZOPHRENIA

The study of psychiatric disorders is notoriously difficult, atypical processing is apparent not just at the cellular level but also at network level, leading to abnormalities pervasive in many higher cognitive functions. In addition to this, the majority of cells of the brain cannot regenerate but rather alter their function via experience driven network integration. For these reasons research tends to be focused on the study of whole organisms, either in patient trials or animal models as opposed to cell cultures. Each mode of research involves drawbacks and benefits which must be carefully assessed.

The fundamental advantage of research in humans is that a diagnosis can be confirmed from the collection of a profile of symptoms from the patient themselves (this is not the case in animal models). Unfortunately, it is not always simple to obtain a complete history from the individual especially given the cognitive symptoms of disorder such as memory and attentional deficits. Another disadvantage is that it may be difficult to gather a large sample of schizophrenia patients who will have a similar experience of the disorder due to its cyclical nature and the heterogeneity of symptoms. Once subjects have been chosen for the study an additional difficulty is ensuring compliance with the testing schedule, whilst controlling for as many lifestyle variables as possible. Antipsychotic medication is also a confounding factor as it is unethical to take patients off of current medication regimes during trials. Additionally, it is not usually possible to conduct research in individuals whilst they are experiencing active phase symptoms and for this reason participants may have to be excluded from the results. Finally, there are limited methods for studying the human brain *in-vivo*. The collection of declarative and behavioural data, as well as drug studies and

fMRI and EEG can provide information regarding the amelioration or deterioration of symptoms but provide little information as to the mechanisms which underlie these processes (Kane and Leucht 2008).

As previously mentioned, it is not possible to make a diagnosis of schizophrenia in an animal using the existing clinical diagnostic criteria. The positive symptoms are so-called as they are additive to typical human experience; as a consequence, there is no way of identifying these hallucinations or delusions non-declaratively (Wong and Josselyn 2016). Given that the positive symptoms can be pharmacologically alleviated in most cases this means that the need for understanding the negative and cognitive symptoms is paramount. Fortunately these aspects of the disorder are easier to model in animals and the results of these investigations can be used to better our understanding of the human condition. Much of the gross anatomy and cellular physiology is preserved between the brains of small mammals and humans; this allows scientists to conduct translational research whereby significant results in animal studies can be followed up in similar patient studies (Geyer 2008). In the context of schizophrenia and other psychiatric disorders behavioural tests for cognition such as memory or attention are very useful in this respect as they can be translated across animals and humans (Neill, Harte et al. 2014, Pedersen, Sørensen et al. 2014). Since the aetiology of schizophrenia is poorly understood it is difficult to mimic all aspects of the disorder at once in an animal model. Broadly speaking there are four different methodologies which are used to model schizophrenia experimentally, most commonly in rodents. These being early life adversity models, drug-induced models, lesioning models targeting particular brain areas or genetic models, a selection of which are summarised in Table 1. There are a number of criteria against which these models are assessed; an ideal model must demonstrate face, construct and predictive validities (Wilson and Terry 2010). Face validity means a model must mimic core symptoms of the disorder; positive, negative and cognitive. Construct validity implies the model has the same underlying cause as the disorder; this is of course problematic when aetiology is unknown. Predictive validity means the model must demonstrate the efficacy of drugs, both current and novel. Schizophrenia studies will often use more than one methodology in order to account for the different strengths and weaknesses of the models (Gaskin, Alexander et al. 2014). The limitations of using animals are fairly evident, not only are there ethical considerations but also concerns relating to whether any significant effects will persist when translated from animals into humans.

Table 1- Animal models of schizophrenia

Model methodology	**Examples**	**Benefits**	**Limitations**
Developmental/ Early life adversity	**Post-weaning social isolation** (McIntosh, Ballard et al. 2013) **Prenatal exposure to Methylooxymethanol acetate (MAM)** (Li, Gulchina et al. 2017) **Prenatal stress** (Ratajczak, Kus et al. 2016)	-Simple to induce. -Provide information regarding the development of the disorder and prodromal phase. -Relatively robust validity demonstrations	-Results are not specific to schizophrenia. -Variability of results and methodologies across different groups.
Drug-induced	**MK-801** (Kawabe and Miyamoto 2008) **Ketamine** (Chindo, Adzu et al. 2012) **PCP** (Seillier and Giuffrida 2016) **Amphetamines** (Peleg-Raibstein, Knuesel et al. 2008)	-The targeting of neurochemical systems whose dysfunction is associated with the disorder (dopamine, glutamate). -Known to induce psychotic-like symptoms in humans, therefore facilitate study of positive symptoms.	-Unlikely to provide information as to the origins of the disorder given that the effects of the drug are irreversible. -Can give false positives in testing efficacy of future drug interventions.
Lesion based	**Neonatal ventral hippocampal lesion** (Tian, Yang et al. 2016) **Mediodorsal thalamus lesion** (Ouhaz, Ba-M'hamed et al. 2017)	-Help to identify brain regions and systems to target for intervention.	-Anatomical abnormalities not usually present at initial stages of disorder.
Genetic knock-outs and mutations	**Disrupted in schizophrenia 1 (DISC1) gene** (Hikida, Jaaro-Peled et al. 2007) **Reelin gene** (Brosda, Dietz et al. 2011)	-Precise targeting of individual gene dysfunction. -Can be used with other methods to investigate genetic vulnerability.	-Compensatory effects of other genes can occlude effects. -Evidence for small contributions of multiple genes, hard to recreate experimentally.

Drug-induced and lesion-based models primarily mimic the symptomatology of schizophrenia and allow for the testing of novel therapies. These methodologies however have limited construct validity meaning that they are unlikely to deepen the understanding of the development of the disorder. Early life adversity models on the other hand allow for the study of environmental factors during the time before symptoms fully manifest. An increased understanding of schizophrenia using developmental models may one day allow for the provision of preventative therapies in at-risk individuals before active phase symptoms begin, thus reducing the need for pharmacological intervention. This approach may also be relevant to other psychiatric disorders with a developmental component such as anxiety, depression (Gershon, Sudheimer et al. 2013).

1.3 POST-WEANING SOCIAL ISOLATION

The present study aimed to investigate cognitive symptoms and specifically the attentional system in schizophrenia. For this reason an early life adversity model was chosen to investigate how rearing conditions can contribute to attentional issues in early adulthood. The selection of a minimally invasive paradigm allows for a more physiological induction of deficits, though the results may not be as marked as with more extreme animal modelling methodologies. Early life stresses contribute to numerous alterations in brain development and are correlated the development of neuropsychiatric disorders later in life and generalised deficits in cognitive function (Aas, Steen et al. 2012). Studies have shown that chronic stress can lead to decreased function of the LC-NE system evidenced as a decreased firing rate (Bravo, Torres-Sanchez et al. 2014).

The model used in study was the post-weaning social isolation rearing (SIR) model of schizophrenia. The rationale for using the SIR model was that, like humans, rats are social animals (Vanderschuren and Trezza 2014), their wide ranging social tendencies have been repeatedly demonstrated. These tendencies include social play, mutually beneficial cooperative behaviours, empathy and the establishment of hierarchy to name a few (Whishaw and Kolb 2005, Saito, Yuki et al. 2016). If rats are prevented from engaging in normal socialisation during their development then significant changes in neurophysiology will ensue in later life.

The effects of social isolation have been studied for over 50 years in neuroscientific and psychiatric research. SIR has been effective in mimicking behavioural (Wilkinson, Killcross et al. 1994), neurochemical (Toua, Brand et al. 2010) and structural symptoms (Bianchi, Fone et al. 2006) of

psychiatric disorders (Lapiz, Fulford et al. 2001, Jones, Watson et al. 2011) as well as responding effectively to current antipsychotic medications (Ko and Liu 2015). These measured changes are translational to the symptoms of the human mental disorder schizophrenia (Mouri, Nagai et al. 2013) as well as depression which is often comorbid with schizophrenia (Samsom and Wong 2015).

The general procedure for SIR is as follows. After weaning, rats are moved from housing with the dam and siblings to experimental housing conditions, group housing for the control animals or solo housing for the experimental animals. Animals remain in these conditions for a number of weeks so that robust differences can be established. Numerous studies have been conducted with minor alterations to the methodology to identify how best to implement these changes. A sample of SIR studies from the last 20 years are summarised in Table 2 to give some idea of methodological variability. SIR has been most often performed using rats as the subject though isolation effects have been demonstrated in a variety of species from monkeys (Washburn and Rumbaugh 1991) to voles (Peuler, Scotti et al. 2012). The impact of SIR on different rat strains is also well documented, each with subtle susceptibilities to different deficits (Weiss, Di Iorio et al. 2000). The strains most commonly used are Lister Hooded, Wistar and Sprague Dawley (S-D). With regard to inclusion of the sexes in SIR studies, more often than not females are excluded from the literature. Justification of this is often not given or simply involves the citation of a previous study which used only males (Ryu, Yoo et al. 2009). Schizophrenia has equal prevalence in men and women so this reasoning is inadequate (Aleman, Kahn et al. 2003). Sex difference findings in SIR studies may help to better understand the role of the endocrine system in the symptomatology of schizophrenia (Weintraub, Singaravelu et al. 2010, Sarkar and Kabbaj 2016). Another methodological difference between papers is the duration of the isolation period. Studies have investigated the impact of short and longer term isolation as well whether resocialisation following isolation is effective (Meng, Li et al. 2010). Typically animals spend 8 weeks in housing conditions before behavioural testing takes place (Witten, Oranje et al. 2014). In a study on shorter isolation durations it was found that behavioural deficits were evident after 4 weeks of isolation but not 24 hours (Seffer, Rippberger et al. 2015). One final point of consideration is the number of animals in the social housing group, this number seems to be highly variable ranging from two per group (Chang, Liu et al. 2014) up to twelve per group (Melendez, Gregory et al. 2004) with an average of around 5 per group (Fabricius, Steiniger-Brach et al. 2011).

SIR studies tend to have a behavioural component in order to demonstrate that symptoms of the disorder have been replicated (face validity). SIR studies will often also include some tests for

neural function either utilising *in-vivo* techniques or neurochemical assays post mortem. Studies such as these help to elucidate the mechanisms underlying dysfunction associated with the disorder which gives rise to the behavioural changes induced. Hence these studies have the potential to provide construct validity. The final criterion required for a successful animal model is predictive validity. Existing schizophrenia medications have been shown to be effective in reversing isolation deficits (McIntosh, Ballard et al. 2013, Ko and Liu 2015). Many novel compounds are also currently being tested in the SIR model (Möller, Du Preez et al. 2013), and if significant benefits are demonstrated these may be followed with clinical trials. The summation of these validities makes SIR a desirable paradigm for modelling schizophrenia.

1.3.1 BEHAVIOUR

The present study investigated behavioural and neurochemical changes induced by SIR that are associated with the negative and cognitive symptoms of schizophrenia. The most commonly reported findings from SIR are hyperactivity, anxiety, neophobia, aggression and deficits in sensory motor gating amongst others (Fone and Porkess 2008). In 2004 the MATRICS initiative established a framework for this kind of research and identified the following as key domains for research: speed of processing, attention/vigilance, working memory, verbal learning and memory, visual learning and memory, and reasoning and problem solving. Behavioural tests in SIR studies are often designed to test these deficits, though often a single behavioural test will have implications for more than one domain. One crucial factor that must be borne in mind when designing SIR behavioural studies is that repeated handling of isolated rats can reverse changes induced by the paradigm. It has been shown that more than one day of handling per week can delete the effects of the model (Holson, Scallet et al. 1991). For this reason it is not advisable to perform numerous tests for different dysfunctions in the same animals. It also limits the usefulness of tests with a lengthy training period. Optimal behavioural tests for SIR studies are therefore minimally invasive and measure the animals' reaction to situations which can be paralleled in human studies. In literature on the SIR model the hyperactivity of isolated animals relative to socialised controls is often referred to. The table below summarises the findings of a number of SIR studies and found this effect to be largely strain and time dependent. Where it is demonstrated, isolation-induced hyperactivity has been shown to be reversible following the application of existing antipsychotic treatments such as risperidone and haloperidol (Fabricius, Helboe et al. 2011, McIntosh, Ballard et

al. 2013). The attenuation of hyperactivity in isolates has also been demonstrated by novel treatments for example cariprazine a partial D_2/D_3 receptor agonist, which adds to the predictive validity of the model and supports translational research (Watson, King et al. 2016).

Table 2- SIR methodology and locomotor activity

The table summarises a sample of SIR rat studies from the last 20 years. The following methodological parameter are indicated: the sex and strain of the animals, how long they were placed in housing conditions for, how long behavioural recordings were made of locomotor activity in an open field and finally whether either group demonstrated hyperactivity during this behavioural recording (ns= no significant difference).

Group	**Sex**	**Strain**	**SIR induction duration**	**Open field test duration**	**Hyperactive group**
(Rosa, Silva et al. 2005)	Male	Wistar	p21 + 10 weeks	5 mins	ns
(Raz 2013)	Male	Wistar	p56 + 2 weeks	5 mins	Isolated rats
(Gentsch, Lichtsteiner et al. 1981)	Male	Wistar	p19 + 5-12 weeks	24 hours 84 hours	ns Socialised rats
(Simpson and Kelly 2012)	Male	S-D	p46 + 3 weeks	5 mins	ns
(Ishikawa, Ogawa et al. 2014)	Male	S-D	p14 + 7 weeks	5 mins	Isolated rats
(Liu, Wang et al. 2017)	Male	S-D	p21 + 4 weeks +5 days resocialise	10 mins	Isolated rats
(Varty, Paulus et al. 2000)	Male	S-D	p21 + 8 weeks	60 mins	ns
(Weiss, Pryce et al. 2004)	Male + Female	S-D	p21 + 13 weeks	60 mins	ns
(Cain, Mersmann et al. 2012)	Male	S-D	p21 + 5 weeks	6 x 60 mins	ns
(Jones, Brown et al. 2011)	Male	Lister Hooded	p23 + 6 weeks	60 mins	Isolated rats
(Watson, Marsden et al. 2012)	Male	Lister Hooded	p23 + 5 weeks	60 mins	Isolated rats
(Zamberletti, Viganò et al. 2012)	Male	Lister Hooded	p21 + 5 weeks	60 mins	Isolated rats
(Witten, Oranje et al. 2014)	Male	Lister Hooded	p25 + 8 weeks	60 mins	Isolated rats
(Einon and Morgan 1978)	Female	Lister Hooded	p23 + 14 weeks	60 mins	Isolated rats

Other than hyperactivity in isolated animals, deficits in paired pulse inhibition (PPI) tests are another common behavioural finding in SIR (Wilkinson, Killcross et al. 1994, Weiss, Di Iorio et al. 2000). PPI testing measures the reaction to two stimuli and gives an indication of sensorimotor gating and attentive mechanisms (Alsene and Bakshi 2011). The first stimulus is sub-threshold and insufficient to induce a startle, whereas the second stimulus is of a larger scale. In normal subjects the first stimulus primes the subject so that the response to the second stimulus is attenuated compared to if the second larger stimulus was presented without a smaller priming stimulus. This attenuated response is not seen in schizophrenia patients indicating adaptive abnormalities. PPI deficits evidenced in schizophrenia are similar to findings in isolated rats, this makes it an ideal translational paradigm (Swerdlow, Weber et al. 2008). One particular study of interest investigated the role of the LC in PPI, though not in the context of SIR. It was found that deficits in PPI were evident after the LC was activated pharmacologically. In the same study this effect was reversed in the presence of atypical antipsychotics (Alsene and Bakshi 2011). Another translational test for sensorimotor gating is latent inhibition which provides an indication of associative learning abilities. Similar deficits in this test have been demonstrated in schizophrenia patients (Gray, Pilowsky et al. 1995) and isolated male rats (Marriott, Tasker et al. 2014).

The response to a novel environment is typically different between socialised and isolated animals. Isolates tend to display hyperactivity in response to novelty, along with differences in measures of anxiety, exploration and investigation. Indications of anxiety are often evidenced as reduced exploration and an unwillingness to venture into open areas in open field tests (Hall, Huang et al. 2000) and the elevated plus maze (Hellemans, Benge et al. 2004). Exploration in response to novelty is purported to be a function of the time period chosen for isolation (Arakawa 2005). Some studies report neophobia (fear of novelty) (Holson 1986) as one of the effects of social isolation and others term differences as an enhanced novelty response. In one study it was found that neophobic tendencies could be reversed depending on lighting conditions (Hall, Humby et al. 1997). Taken together these results highlight that novelty response is certainly affected in SIR, however this is not a unidirectional phenomenon. This highlights that attributing broad descriptions of behaviour may be problematic in SIR and the need for reporting findings in a specific context, as novelty can refer to any number of stimuli. One fairly consistent novelty finding is that isolated animals take longer to habituate to new settings (Paulus, Bakshi et al. 1998, Powell, Swerdlow et al. 2002).

The novel object recognition test is a test regularly used in SIR studies. This is a useful translational test as healthy humans and rats are drawn to explore novel objects as opposed to familiar ones (Lyon, Saksida et al. 2012). In schizophrenia in humans and in translational animal models this trait

is altered and subjects will not spend significantly longer exploring novel objects (Young, Powell et al. 2009). In this test animals are presented with two similar objects for a period of time and after an interval one of these original objects will be presented along with a novel object. The response to this novel object has implications for salience processing as well as short term memory function depending on the inter trial delay. Deficits in the ability to distinguish between the two objects have been found in both male (Jones, Brown et al. 2011) and female rat studies (McLean, Grayson et al. 2010). It has been found that isolated animals have significantly impaired memory performance in the NOR test when the inter-trial interval is increased. The ability of isolates to discriminate between the novel and familiar object has been shown to be attenuated after time delays of 60 minutes (Bianchi, Fone et al. 2006) and 4 hours (McLean, Beck et al. 2008). The hippocampus plays a critical role in novel object recognition as lesioning causes specific deficits in task performance (Ainge, Heron-Maxwell et al. 2006). Sex hormones have also been shown to play a role in regulating the function of the hippocampus in NOR tests in female rats. Elevated levels of endogenous hormones progesterone and estradiol are correlated to improved performance scores in the NOR test (Tuscher, Fortress et al. 2015).

Social withdrawal is one of the negative symptoms of schizophrenia and this can be tested in animal model using ultrasonic vocalisation and social interaction studies. Adult rats emit vocalisations at two key frequencies, 50 kHz and 22 kHz which are suggested to be indicative of positive and negative affective states respectively (Portfors 2007, Burgdorf, Kroes et al. 2008). Vocalisations at the 50 kHz range are thought to be indicative of positive affect, in a study it was found that after recordings of 50kHz pro-social calls were played, isolated rats significantly decreased their motor activity, indicating an abnormal response to social stimuli (Seffer, Rippberger et al. 2015). This effect was evident in longer term isolated rats but not in rats isolated for 24 hours, no differences were found in response to playback of 22 kHz recordings. In a similar study it was found that male isolates emitted fewer pro social 50 kHz calls in total than their socialised counterparts and in addition to this showed no difference in their call response to sexually receptive and non-receptive females (Inagaki, Kuwahara et al. 2013). Taken together these results imply abnormal response in social environments which may be of relevance to schizophrenia. Vocalisations at the 22 kHz range are thought to be indicative of negative affect, a study investigating the differences between long and short term isolation showed that 24 hour isolation was accompanied by an increase in the number and duration of calls in this frequency range. Contrastingly after a longer 2 week isolation the number of calls was reduced (Tomazini, Reimer et al. 2006). Another study found that animals which had undergone isolation made more

vocalisations at 22 kHz in response to an aggressor (Von Frijtag, Schot et al. 2002). Though the number of calls made by isolates does not consistently decrease or increase in these different studies, they nonetheless provide useful data on the way in which social function is abnormal after SIR. On account of their lack of normal social play isolated rats, have also been found to have heightened aggressive responses to other rats (Wongwitdecha and Marsden 1996). The wide ranging deficits that are induced by SIR provide incentive for the follow up of behavioural test with neurochemical analyses to probe the systems which may underlie these deficits.

1.3.2 NEUROCHEMISTRY

As well as behavioural data there is much evidence to support that SIR is also able to induce neurochemical changes in a variety of brain areas. Many studies have found evidence for dopamine dysfunction which parallels schizophrenia. These studies target metabolism, turnover, receptor populations and overall concentrations of neurotransmitter to provide information on mechanistic differences. When an *in-vivo* microdialysis study was performed, amphetamine-stimulated DA release was found to be reduced in isolated rats in the prefrontal cortex but not in the nucleus accumbens, this result fits conveniently with the hypofrontality theory of dopamine in schizophrenia and reinforces SIR as an appropriate modelling paradigm (Fabricius, Steiniger-Brach et al. 2011). In another *in-vivo* microdialysis study differences in amphetamine-stimulated release were found in the prefrontal cortex. Serotonin (5-HT) release was decreased in the prefrontal cortex of isolates but no differences were found for DA (Dalley, Theobald et al. 2002). A study investigated DA and 5-HT in post mortem tissue obtained from isolated rats. In the hippocampus it was found that the concentration of 5-HT was reduced and 5-HT turnover was increased. In the ventral striatum 5-HT concentrations were increased in isolated animals. In the hippocampus DA turnover was increased but no change in concentration was evident and in the ventral striatum no differences were found between the groups for DA turnover or concentration (Brenes and Fornaguera 2009). Glutamatergic neurochemistry is less well studied in the SIR model. One study explored alterations in glutamate function and found that the ability of a metabotropic glutamate receptor agonist to increase extracellular glutamate in isolated animals was attenuated in addition to various differences in metabotropic glutamate receptor subtype populations (Melendez, Gregory et al. 2004). Additionally a metabotropic glutamate receptor agonist was shown reverse hyperactivity and novel object discrimination impairments in isolated animals. In group animals the same agonist

reduced novel object discrimination abilities (Jones, Brown et al. 2011). Though there is evidence for NMDA receptor dysfunction in schizophrenia, few studies have sought to characterise these populations in SIR.

Genetic and brain imaging studies have also revealed functional and anatomical differences in socially isolated rats. Techniques such as magnetic resonance spectroscopy (MRS), magnetic resonance imaging (MRI) and positron emission tomography (PET) enable measurements of the metabolic activity of the brain. Microarray and reverse transcription polymerase chain reaction (RT-PCR) techniques allow for the quantification of gene expression of tissues *in-vitro*. These studies further implicate certain brain regions as being vulnerable to SIR. In particular, it has been shown that there is decreased flurodeoxglucose uptake in the hippocampus of isolates which is used as a marker of metabolic activity as measured with PET (Bonab, Fricchione et al. 2012). In accordance with this, glutamate and glutamine concentrations have also been found to be reduced in the hippocampus when measured with MRS (Shao, Yan et al. 2015), these results provide potential translational biomarkers for the human disorder. In a RT-PCR study measuring glutamate and GABA receptor subunit expression in distinct regions of the hippocampus isolated animals were found to have attenuated $GABA_A$ receptor expression in the dentate gyrus but increased glutamate NMDA and kainate receptor expression in the CA1 and CA3 regions of the hippocampus (Iwata and Yamamuro 2016). Using microarrays it has been found that the PFC demonstrates decreased immediate early gene expression (Levine, Youngs et al. 2007) which is linked to the disruption of learning and memory processes. Additionally it has been shown that in post-mortem tissue there is an overall reduction in PFC volume associated with SIR which corresponds to changes seen in schizophrenia though this is not accompanied by a decrease in neuron number (Day-Wilson, Jones et al. 2006). This PFC volume reduction has also been demonstrated utilising MRI (Schubert, Porkess et al. 2009).

With regard to NE and SIR, the relationship between the two has been relatively well studied in the context of ethanol intake rather than schizophrenia. These studies still provide relevance for schizophrenia however because of the high comorbidity of alcohol abuse disorder and schizophrenia. It was demonstrated in a microdialysis study that ethanol administration causes an increase in extracellular DA and NE in the nucleus accumbens (an area associated with addictive behaviours) in isolated animals (Karkhanis, Locke et al. 2014). In this study there was no difference found in DA and NE levels prior to alcohol administration. Dopamine beta hydroxylase (DBH), the enzyme which converts DA to NE, was stained in various brain areas in an SIR study. It was found that the density of DBH axons was greater in in the infra-limbic region of the PFC in isolates

(Kuramochi and Nakamura 2009). NE levels were found to be reduced in the ventral striatum in isolates (Brenes, Rodríguez et al. 2008) when assayed with HPLC. A reduction in the density of noradrenergic neurons in the both the dorsal and ventral prefrontal cortex was evident in isolates, no differences were found in the hippocampus (Ishikawa and Ishikawa 2013). *In-vitro* measurements revealed no differences in basal NE release in isolates or in response to high potassium stimulation in the hippocampus (Fulford and Marsden 1997). The same test in the presence of idazoxan, an α_2-adrenoceptor antagonist, caused a greater increase of NE release in isolates than in socialised animals. This study commented that isolation may have enhanced α_2-autoreceptor function on the presynaptic terminal. This lab also performed *in-vitro* and *in-vivo* assays on a number of other neurochemical systems in the SIR model which demonstrates the variable susceptibility of different neurotransmitters and brain regions to the paradigm. Elevated and prolonged presynaptic dopamine transmission in the nucleus accumbens was evident in isolates following a foot shock exposure during an *in-vivo* microdialysis study (Fulford and Marsden 1998) this result was found to be replicable (Hall, Wilkinson et al. 1998). The release of presynaptic 5-HT was also increased in the nucleus accumbens of isolated rats (Fulford and Marsden 1998). This work was supported with a similar *in-vivo* study addressing the interaction of dopamine and 5-HT in the nucleus accumbens through the inhibition of dopamine synthesis by a pharmacological agent (Fulford and Marsden 2007). It is worth noting that the insertion of the microdialysis probe in these in-vivo experiments is likely to have caused significant stress to both groups which may have masked additional effects of the isolation paradigm (Fulford and Marsden 1998, Fulford and Marsden 1998). A separate study measured NE uptake as well as adrenoceptor binding profiles in a variety of brain regions (Kraeuchi, Gentsch et al. 1981). In the hypothalamus, uptake of NE into the synaptosomes was increased in isolates. A β-adrenoceptor binding ligand was used in the same areas and in the thalamus and medulla-pons binding was reduced in isolates, when an α-adrenoceptor binding ligand was used; binding was shown to be increased in the medulla-pons in isolates.

An additional study used an α-adrenoceptor antagonist (ORM-10921) in an SIR study to assay its ability to improve scores in variety of behavioural test. ORM was found to improve the scores of isolated animals in PPI testing and recognition memory tests (Uys, Shahid et al. 2016). These studies implicate altered adrenoceptor function in SIR which may be relevant to schizophrenia. No concentration differences were found when NE and 5-HT were measured post mortem using HPLC in the hippocampus, however the turnover of 5-HT was significantly increased in isolates, neurotransmitter concentrations were not found to be correlated to activity in the forced swim test

(Brenes, Rodríguez et al. 2008). In a follow up study the same group measured NE and 5-HT this time in the ventral striatum and prefrontal cortex, no significant differences were found in the prefrontal cortex but in the ventral striatum NE was significantly reduced in isolates (Brenes, Padilla et al. 2009).

Previously SIR studies have utilised ELISAs to investigate the abundance of BDNF (an indicator of stress and depressive mechanisms) in the SIR model. In the hippocampus of isolated male S-Ds it was found that BDNF levels were reduced compared to socialised animals, (Scaccianoce, Del Bianco et al. 2006, Pisu, Garau et al. 2016). A separate study found this effect in isolates females (and not males) but only in the CA3 hippocampus region (not CA1)(Weintraub, Singaravelu et al. 2010). In the prefrontal cortex BDNF levels were elevated in isolated male Wistar rats (Shao, Han et al. 2013), a visually similar (non-significant) trend was seen in male S-Ds (Scaccianoce, Del Bianco et al. 2006), no differences were found in the nucleus accumbens and striatum in these studies. Another group found no differences between isolated and socialised rats when testing with ELISA for corticosterone levels (also associated with stress) in blood plasma in male S-Ds (Zhang, Wang et al. 2014). It is clear that SIR is able to induce not only behavioural deficits but also changes in neurochemistry. This body of research is being consistently added to which will allow for a more holistic understanding of the neural systems which are vulnerable during development.

1.4 RATIONALE

The implications of schizophrenia on society and the individual are severe. There is a pressing need for improved treatment options particularly for cognitive and negative symptoms. Such improvements would allow those with a diagnosis of schizophrenia to continue to work and live independently and facilitate societal reintegration. The neural substrates of these symptoms must be investigated in order to develop effective treatments for their alleviation. There is extensive literature regarding the attentional system and its regulation by the LC-NE system in healthy individuals. There is also substantial evidence that one of the cognitive symptoms of schizophrenia is abnormal attentional processing. So far in the literature there have been few attempts to draw together the dysfunction of the attentional system and its regulation by LC-NE in the context of schizophrenia, specifically using the SIR model. Behavioural studies of attention in models of schizophrenia are not always followed up with neurochemical analyses. The present study was

designed to address this gap in the literature and provide novel evidence which may be of clinical relevance. The study will also provide additional data on social function which pertains to the negative symptoms of schizophrenia. This study will include equal numbers of male and female rats in order to investigate sex differences; this is relevant as females may respond differently to SIR (Sutcliffe, Marshall et al. 2007, Hong, Flashner et al. 2012), and are often excluded from studies.

Recently in psychiatry there has been a move away from the rigid categorisation of disorders and research pertaining only to a single mental disorder. This is due to the lack of consistently efficacious treatments across populations as well as the high prevalence of cross-over diagnoses and also because current definitions are not necessarily facilitating treatment but perhaps, rather adding to stigmatisation. In 2002 in Japan the diagnostic term for schizophrenia was changed from 'split mind disorder' to 'integration disorder' in order to address societal prejudice and reflect a more modern understanding of schizophrenia (Sato 2006). Therefore, although this project is investigating a particular system (attention) in the context of a particular disorder (schizophrenia) this research may be relevant to many other people beyond those with a diagnosis of schizophrenia. This work will therefore reinforce the validity of SIR as a model for schizophrenia or, if the results do not translate into the human disorder, provide valid data about the effects of isolation on the attentional system in a non -schizophrenic context (Samsom and Wong 2015).

1.5 AIMS AND HYPOTHESIS

The aim of this study was to use the SIR model to provide a better understanding of the undertreated cognitive and negative symptoms of schizophrenia. In particular the attention dysfunction in schizophrenia was of interest when planning this study. The experiments were designed with the primary aim of probing attention from a behavioural and neurochemical perspective and to utilise existing knowledge on the attentional system of the brain in the context of schizophrenia. The secondary aim of the study was to measure social function. This study also aimed to address how the SIR paradigm might differentially affect males and females.

This study hypothesised that NE dysfunction underlies attentional issues associated with the disorder of schizophrenia. It is also hypothesises that early life adversity in the SIR paradigm will be able to serve as a model of these systems. In line with the primary aims of the study we expected

to find behavioural deficits in isolated animals such as hyperactivity and abnormal attention towards novelty in line with existing literature. We hypothesised that attentional abnormalities in isolated animals may be accompanied by attenuation in the functional release of NE or total NE concentration in certain brain areas. In line with the secondary aims of the study we hypothesised that social isolation may affect calling behaviours. Finally, we expected to find behavioural and neurochemical differences between the males and females.

1.6 OBJECTIVES

The primary aim, *to assess the behavioural and neurochemical aspects of the attentional system in the SIR model and progress understanding of the cognitive symptoms of schizophrenia*, was carried out with the following objectives:

1.1) Use *in-vivo* novel object recognition testing to assess attentional processing and measure response to novelty (environment and objects), locomotor activity, exploratory and anxiety-like behaviour in socialised and isolated, male and female animals.

1.2) Use *in-vitro* superfusion technique to assess relative functional release of NE when stimulated with glutamate in locus-coeruleus projection areas hippocampus and prefrontal cortex in socialised and isolated, male and female animals.

1.3) Use *in-vitro* superfusion technique to assess relative functional release of NE when stimulated with glutamate in presence of CNQX and MK-801 (ionotropic glutamate receptor antagonists for AMPARs and NMDARs respectively) in locus-coeruleus projection area hippocampus in socialised and isolated, male and female animals.

1.4) Use *in-vitro* superfusion technique to assess relative functional release of NE when stimulated sequentially with glutamate and then GABA (or vice versa) followed by high potassium stimulation in locus-coeruleus projection areas hippocampus and prefrontal cortex in socialised and isolated, male and female animals.

1.5) Use *in-vitro* ELISA and BCA assay to quantify absolute concentrations of glutamate and NE in locus-coeruleus projection areas hippocampus and prefrontal cortex in socialised

and isolated, male and female animals, expressed as concentration of neurotransmitter per wet weight of tissue and as concentration of neurotransmitter per total protein.

The secondary aim, *to assess social dysfunction in the SIR model in order to progress understanding of the negative symptoms of schizophrenia*, was carried out with the following objective:

2) Use *in-vivo* ultrasonic vocalisation testing to investigate calling behaviour associated with negative affect after exposure to novel environment in socialised and isolated, male and female animals.

CHAPTER 2
-METHODOLOGY-

2.1 EXPERIMENTAL CONDITIONS

2.1.1 ANIMAL REARING

This project was approved by the University of Cape Town health sciences faculty animal ethics committee (UCT HSF AEC), project number: 047-2014 (Figure 69, Figure 70). All experiments were carried out in accordance with South African National Standards (SANS: The care and use of animals for scientific purposes, 2008). In this project 176 outbred Sprague-Dawley (S-D) rats (male n=76, female n=100) were used. The use of animals in this study was necessary in order to model a complex mental disorder in a whole organism. All animals were issued from the UCT research animal facility. Day of birth was considered as post-natal day one (p1). Litters were culled to six or eight pups of a single sex, as determined by gonadal anatomy (Torgrimson and Minson 2005), on p7 to ensure optimal nutrition from the dam for each of the pups. A litter of six or eight pups, alternating between males and females, was collected each week and brought to the Human Biology Satellite animal facility on p21 to be housed until end point. On their day of arrival pups were weighed and randomly assigned to housing groups. From each litter of six or eight, three or four pups were housed together in a 'socialised' setting and the other three or four pups were put into individual 'isolated' housing. A total of twenty two litters were used in this study. The final numbers for the groups were as follows; female socialised (FS) n=50, female isolated (FI) n=50, male socialised (MS) n=38 and male isolated (MI) n=38. All animals were housed in the same room with 12 hour/12 hour reversed light/dark cycle (06h00-18h00 dark, 18h00 -06h00 light). 'Rat Chow' food (dry pellets) and tap water were provided *ad libitum*. Light intensity in the facility was maintained at 150-200 lux so as not to damage the rat visual system; albino rats have been found to develop phototoxic retinopathy at levels above 325 lux (Bellhorn 1980). The room was well-ventilated and temperature was maintained at 21-24 °C (checked daily). Welfare monitoring of each rat took place every day to check for signs of discomfort, stress and physical deterioration. In accordance with AEC guidelines, any animals showing signs of distress were decapitated immediately. This was necessary only for one animal (KGFI15) during the course of the project on 04/06/15 after physical deterioration was evident one week after the animal had arrived in the

Satellite facility. The animal was showing signs of severe weight loss compared to littermates at p28; this was reported to the UCT HSF as required. Animals were housed in translucent Plexiglas cages with wire lids and sawdust for bedding, as it has been shown that wired flooring in the cages can act as an additional stressor (Weiss, Feldon et al. 1999). Animal bedding was changed and cages cleaned at least once or at most twice weekly according to housing group, body weight of each animal was recorded once weekly during cleaning. Cage sizes were as follows; extra small (30 cm l x 15 cm w x 15 cm h), small (35 cm l x 20 cm w x 15 cm h), medium (40 cm l x 20 cm w x 20 cm h), large (55 cm l x 35 cm w x 20 cm h). The rats were moved to increasingly large cage sizes in line with the stage of growth of the animals and to allow for comfortable perambulation (Table 3). All animals were housed in large cages prior to behavioural testing during p78-82. End point by decapitation occurred during p90-94, (Figure 1).

Table 3- Progressive cage sizes for different housing conditions

Post-natal week	**Cage size** **Socialised rats (3/4 per cage)**	**Cage size** **Isolated rats (1 per cage)**
p21-28	Extra small	Extra small
p28-35	Small	Extra small
p35-42	Small	Small
p42-49	Medium	Small
p49-56	Medium	Medium
p56-63	Large	Medium
p63-End point	Large	Large

2.1.2 EXPERIMENTAL DESIGN

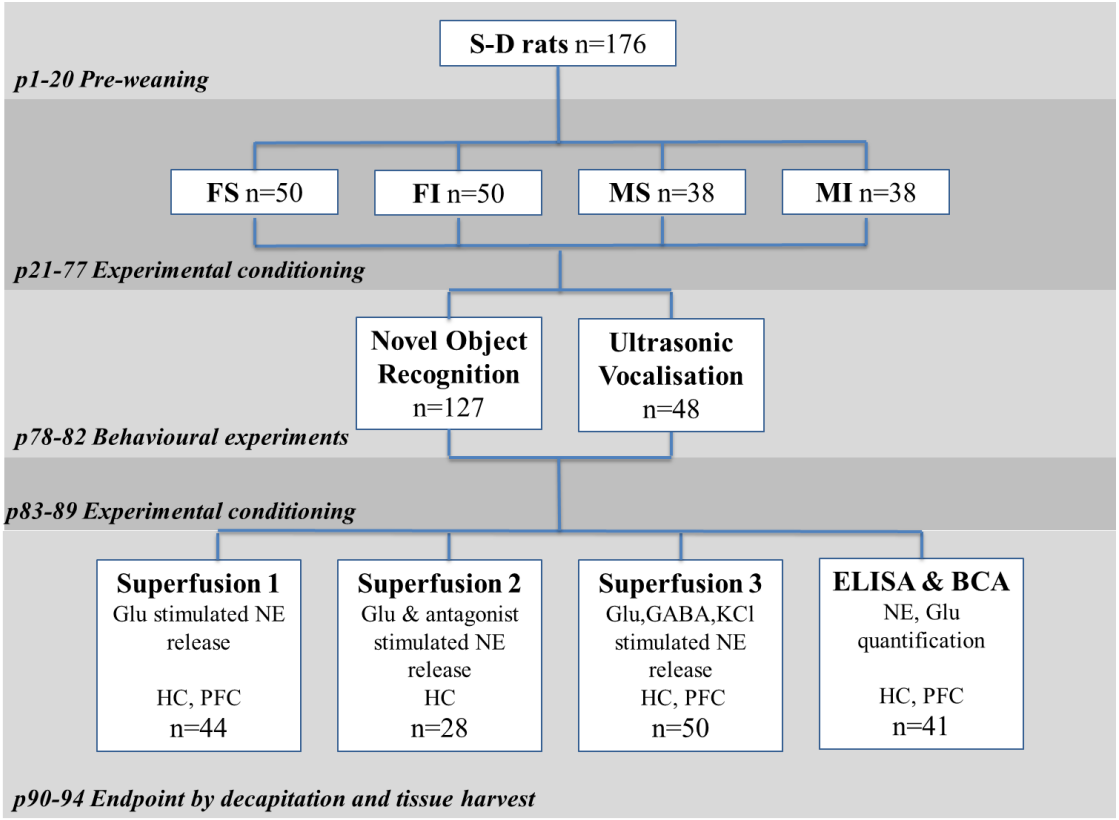

Figure 1- Experimental design

During the pre-weaning phase Sprague-Dawley (S-D) rats were housed in litter groups with dam. During experimental conditioning rats were split into testing groups; female socialised (FS) and female isolated (FI), male socialised (MS), male isolated (MI). During behavioural experiments rats underwent one of two tests listed and then were placed back into the same housing conditions as before. Rats were killed at p90-94 by decapitation and tissue of interest was rapidly harvested, following this, one of four possible neurochemical analyses was performed. Superfusion (SF) experiments involved stimulating the release of radioactively-labelled norepinephrine (NE) with glutamate (Glu). Superfusions were carried out in prefrontal cortex (PFC) and hippocampus (HC) tissue with the addition of various other compounds; gamma-amino butyric acid (GABA), potassium chloride (KCl) and ionotropic glutamate receptor antagonists CNQX and MK-801. For enzyme linked immunosorbent assays (ELISA), brain areas were frozen after decapitation, and were subsequently used to quantify glutamate and NE concentrations. Bicinchoninic acid (BCA) protein assays were used to measure the protein content in the prefrontal cortex and hippocampus. Of the 176 total animals, 1 animal perished shortly after arrival in Satellite facility. 175 animals were used in behavioural testing; tissue was harvested from all 175 animals however not all of the frozen tissue intended for ELISA and BCA experiments was used due to limited space in the assay plates. Therefore neurochemistry was performed on 163 animals in total.

2.1.3 POST-WEANING SOCIAL ISOLATION REARING

The post-weaning social isolation method used in this project was based on a protocol characterised by collaborators at North-West University, South Africa (Toua, Brand et al. 2010, Möller, Du Preez et al. 2011, Möller, Du Preez et al. 2012, Möller, Du Preez et al. 2013). The only modification was that both male and female rats were used in the present study.

At p21, six or eight rats of the same sex and from the same litter were removed from their home cage where they received nutrition from the dam. Three or four rats were then placed together into 'socialised' housing (sharing one cage) or the other Three or four rats were placed into 'isolated' housing (single rat per cage). The social isolation protocol pertains only to physical social isolation as visual, olfactory and auditory cues from rats were still present in the facility since all animals were housed in the same room. This is more representative of human society as individuals with a diagnosis of schizophrenia may be isolated in terms of physical interactions with other people but are unlikely to be completely cut off from all societal cues. Cages were separated in the room by sex in order to minimise odour cues between male and female mammals which have been shown to have an impact on behaviour (Doty 1986). All animals underwent a strict minimal handling protocol, as it has been shown that if socially isolated rats are handled regularly, the impact of isolation is cancelled (Pritchard, van Kempen et al. 2013). For this reason isolated animal cages were cleaned once a week only and socialised animal cages twice weekly. All animals were weighed once weekly during the cleaning of cages, on a Thursday. Handling of animals was by base of tail only so that animals could become accustomed to being moved by experimenters (Gärtner, Büttner et al. 1980, van Driel and Talling 2005) and so that physical contact leading to bonding could be minimised. In terms of environmental enrichment, socialised animals in large cages were given translucent red tubes as is standard with rat care in our facility, isolated animals received no enrichment as it has been shown that enrichment can diminish effects of social isolation (Tanaś, Ostaszewski et al. 2015).

2.1.4 BEHAVIOURAL EXPERIMENTS

Each animal underwent behavioural testing during p78-82 consisting either of novel object recognition (NOR) testing or ultrasonic vocalisation (USV) testing. Behavioural testing was

performed after animals had undergone 8 weeks of socialisation or isolation, this being consistent with the time window for behavioural testing by other experimental groups (Möller, Du Preez et al. 2013, Hong, Lee et al. 2015). At this time the rats were adults having sexually matured at p45-48 or p32-34 for males and females respectively (Lewis, Barnett et al. 2002). This time window was chosen to parallel the time at which symptoms contributing to a diagnosis of schizophrenia are fully realised in humans, early adulthood (Bergen, O'Dushlaine et al. 2014). Animals were brought to behavioural suite between 07h00 and 09h00, 60 minutes after start of the dark cycle, so as to not impact wakeful grooming and eating, therefore to coincide with the time when rats would naturally be more active and display behaviour after sleeping during light hours (Castro-Faúndez, Díaz et al. 2016). Behavioural tests took place in ≥50 lux red light as it has been shown that rats are more behaviourally active in red light than white light conditions (Hall, Humby et al. 1997). The temperature in the behavioural suite was kept consistent with the housing facility. Animals were transported in their home cage to the behavioural suite outside of the testing room and remained here for at least 60 minutes prior to recordings to minimise stress from transportation. This was also so that the animal undergoing testing was not influenced by other rats in the room. These experiments took place in the level 3 behavioural testing suite of the UCT FHS anatomy building.

2.1.5 NEUROCHEMICAL EXPERIMENTS

At experimental end point (p90-94) rats were killed by rapid decapitation by guillotine and tissue was harvested for neurochemical analysis; for either *in-vitro* superfusion or ELISA. The guillotine was chosen as the method of killing so as to keep the condition of the brain as close to physiological as possible. Therefore blunt force trauma by concussion and the use of anaesthetics was avoided. All tissue was immediately placed post-mortem into ice-cold conditions to reduce the breakdown of monoamines (Kontur, al-Tikriti et al. 1994). The brain areas chosen for investigation in the neurochemical analyses were the hippocampus and prefrontal cortex. Both of these areas receive noradrenergic projections from the locus coeruleus and have been shown to be involved in attentional processing. Human EEG studies have also demonstrated a strong link between activity of the LC-NE system and PFC and HC regions (Berridge and Foote 1991), this supports the translational aspect of this study. These studies will allow for examination of cortical level processing as well as limbic system function. These experiments took place in the level 5 Neuroscience laboratory of the UCT FHS anatomy building.

2.2 NOVEL OBJECT RECOGNITION TESTING

The novel object recognition (NOR) test is used in animal research to measure a variety of behaviours. NOR testing is minimally stressful/ invasive and does not require lengthy training (Grayson, Leger et al. 2015) or positive and negative reinforcement (Antunes and Biala 2012). The test is primarily used to assess memory function (Pyndt Jørgensen, Krych et al. 2015) but also has relevance to other cognitive faculties such as attentional processing and response to novelty (Mansour, Babstock et al. 2003, McLean, Grayson et al. 2010, Rajagopal, Massey et al. 2014). In schizophrenia, cognitive function is commonly impaired and largely untreated (Green 2016) and since the NOR test is able to provide indicators of cognitive function this makes it a useful tool for translational research (Perry, Minassian et al. 2010). This translatability stems from the fact that healthy rodents and humans alike will naturally show preference towards exploring novel stimuli (environments or objects) as opposed to familiar ones (Ennaceur and Delacour 1988, Fone and Porkess 2008). In schizophrenia this preference for novelty has been found to be reduced (Bachiller, Lubeiro et al. 2015, Andersen, Campbell et al. 2016) and a similar finding is evident in NOR testing; isolated animals have a reduced tendency for exploring novel objects (Bianchi, Fone et al. 2006). The test can also provide information about anxiety and movement (Hoffman and Basurto 2014) which is also relevant to the symptomatology of schizophrenia. Isolated animals are commonly found to be hyperactivity in the initial open field phase of the NOR test (Del Arco, Zhu et al. 2004, Ishikawa, Ogawa et al. 2014) which has relevance to disorganised motor behaviour in schizophrenia.

The test took place over two consecutive days and involved three phases (Figure 2). Socialised and isolated animals were recorded in the arena individually. The total number of animals recorded as part of the NOR test was as follows; FS n=34, FI n=33, MS n=30 and MI n=30. Due to technical difficulties not all data were suitable for analysis; final numbers used for each trial phase are reported below. The protocol used in the NOR test followed that of our collaborators at North-West University (Möller, Du Preez et al. 2013, Uys, Shahid et al. 2016). On the first day, each animal underwent phase 1 ‘open field exploration’ for 10 minutes in an empty arena. On the second day, the animal underwent phase 2 ‘object familiarisation’ for 10 minutes whereby two identical objects were introduced to the arena. Finally, after an interval of 60-90 minutes, phase 3 ‘novel object recognition’ took place for 5 minutes where one familiar object was swapped for a novel object.

The apparatus was cleaned with 70 % ethanol and wiped down with water after each rat was tested so as to minimise olfactory confounds. In the present study, the arena consisted of an open-topped black box (60 cm x 60 cm arena and height of 50 cm). A videorecorder was fixed on a frame above the arena to capture the movement of the animal. The videos were later analysed offline using Noldus Ethovision (version 7.0). Movement was tracked with centre-point detection of the rat for more general exploratory parameters and nose-point detection for more specific investigatory parameters. Statistical tests were performed on parameters recorded from the first minute of each video to give an insight into the immediate novelty response. Statistical tests were also performed on parameters recorded from the first five minutes of each video to give an idea of short term attentional processing. Analysis of the full ten minutes of phase 1 was also performed. Analysis of all time bins was beyond the scope of this project.

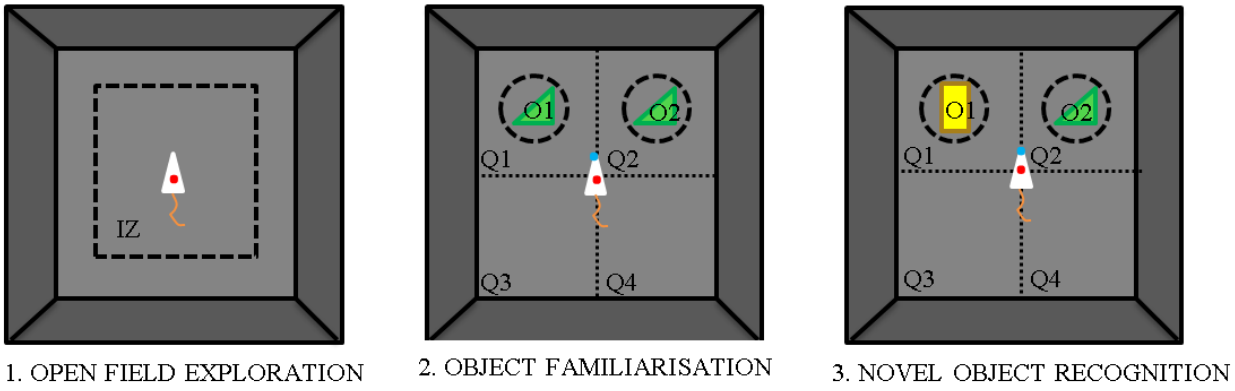

Figure 2- Novel object recognition test apparatus

Three phases of NOR test.

1: Centre-point detection tracking (red dot), measuring distance travelled (cm), time spent (s) within and number of entries into the inner-zone (IZ). 1st minute, 5 minutes and 10 minutes analysed.

2 and 3: Centre-point detection tracking (red dot) measuring distance travelled (cm), time spent (s) within and the number of entries into the quadrants (Q1, Q2, Q3, Q4). Nose-point detection tracking (blue dot) measuring time spent (s) with, the number of approaches to, and latency (s) to the first approach of the objects (O1, O2). 1st minute and 5 minutes analysed.

2.2.1 PHASE 1- OPEN FIELD EXPLORATION

On the first day of testing, after spending 60 minutes in the home cage in the behavioural suite as detailed above each animal underwent phase 1open field testing (Figure 2.1) FS n=24, FI n=23, MS n=25 and MI n=25. During the open field test the arena was completely empty, no objects were present, and each rat was recorded moving around this open space for 10 minutes. This provided a measure of the rat's reaction to a novel environment outside of the home cage for the first time since conditioning and to allow the rat to become familiar with the arena before objects were added in later phases. At the end of this time the recording was stopped and the animal was returned to its home cage.

Phase 1 was considered as a habituation session for the latter stages of the NOR task (Antunes and Biala 2012). This phase exposed animals to a novel environment for the first time since experimental conditioning and allowed them to become familiar with the apparatus before objects were added in the later phases. Animals were tested individually in the arena. The experimenter started the recording, gently placed the rat in the middle of the arena facing the back wall (East) and then exited the room. All rats were held at the base of the tail, in line with the protocol of minimal handling. The following parameters from phase 1 were recorded based on rat centre-point detection; distance travelled (cm), time spent within the inner-zone (s) and the number of entries into the inner-zone. The distance was used as a measure of locomotor activity (Prut and Belzung 2003), and inner-zone measurements were used as an indicator of exploratory behaviour (Matsumoto, Uehara et al. 2014) and anxiety, related to fear of open or unfamiliar territory (Das, Barhwal et al. 2015). Three temporal analyses were conducted for each of these parameters. The analysis of the first minute was performed in order to provide an indication of immediate reaction to the novel environment, the analysis of the first 5 minutes and full 10 minutes of the test were to give an indicator as to whether response changed over time and how the groups might habituate.

2.2.2 PHASE 2- OBJECT FAMILIARISATION

On the second day, after spending 60 minutes in the home cage in the behavioural suite, each animal underwent phase 2 object familiarisation testing (Figure 2.2). FS n=24, FI n=22, MS n=25 and MI n=24. The testing arena was the same as in phase 1 but in phase 2 two identical objects

were added. Object 1 and object 2 (two identical triangular blocks of wood, 5 cm x 5 cm x 3 cm) were secured to the base in the top left hand and top right hand corners of the box. The animal was left to explore these objects for 10 minutes to allow for familiarisation. At the end of this time the recording was stopped and the animal was returned to its home cage. The arena and objects were cleaned with 70 % ethanol solution and water and wiped down in between animals. The objects were swapped from corner to corner in between every trial to eliminate potential bias.

The purpose of this stage of testing was to allow the animals to familiarise themselves with the objects and show that there was no preference for either. If a difference in preference was demonstrated between the two objects this was addressed using a correction factor (Equation 2). The following parameters from phase 2 were recorded based on rat centre-point detection; distance travelled (cm), time spent in each quadrant of the arena (s), and the number of entries into each quadrant. The following parameters from phase 2 were recorded based on rat nose-point detection; time spent (s) with the identical objects, the number of approaches to the identical objects and the latency to approach each identical object for the first time (s). The latency to enter the quadrants was not measured as this was subject to random error since the animals were placed in the centre of the testing arena at the intersection of the four quadrants at the start of the experiment. One minute and five minute analyses were performed on data.

When the raw data was analysed, it was found that for some variables, animals were showing a preference for one of the objects or quadrants and these results were consistent in both the one minute and five minute analyses (Appendix Table 40), these data were then corrected to adjust for bias (Equation 2). During testing all efforts were made to standardise the apparatus including cleaning and swapping the objects in between trials, and by using overhead lighting to eliminate shadows. There may however have been stimuli remaining in the room which the rats were sensitive to (but the experimenters could not detect), for example a smell or subtle visual cue. Another researcher in the same laboratory also found a similar preference for one of the objects when analysing phase 2 of the novel object test (data unpublished). It is therefore recommended that the equipment be moved to another room for future study in order to determine the source of the bias.

2.2.3 PHASE 3- NOVEL OBJECT RECOGNITION

This phase of testing also took place on the second day. After phase 2 each rat was returned to its home cage for 60-90 minutes. After this time had elapsed each animal underwent phase 3 novel object recognition testing (Figure 2.3) FS n=24, FI n=23, MS n=25 and MI n=24. During phase 3 testing object 1 was replaced with a rectangular block of wood (5 cm x 2 cm x 2 cm), serving as a novel object and object 2 was kept the same as in the previous trial, serving as a familiar object. The animal was left to explore these objects for 5 minutes. The arena and objects were wiped down with 70 % ethanol solution and then water and in between animals.

The purpose of this phase of testing was to determine whether the animals showed a preference for the novel object. Previous studies have demonstrated that socialised animals spend significantly longer with the novel object whilst isolated animals show no preference for either of the objects (Bianchi, Fone et al. 2006). The parameters recorded were the same as those in phase 2. In this phase a discrimination index was calculated to quantify preference for the novel object. The difference in time spent in Quadrant 1 and Quadrant 2 (s) was divided by the total time spent in these two quadrants (Equation 1) (Tian, Pan et al. 2015). A discrimination index was also calculated in this way for the time spent (s) with Object 1 and Object 2. One minute and five minute analyses were performed on data.

Equation 1-Calculation of discrimination index form phase 3 NOR variables

$$\frac{(Q1 - Q2)}{(Q1 + Q2)}$$

2.3 *IN-VITRO* SUPERFUSION

In-vitro superfusion experiments involved incubation of brain tissue with a radioactively-labelled neurotransmitter, which was taken up into the synaptic terminals. The tissue was perfused with artificial cerebrospinal fluid (Krebs buffer) to maintain neural function and then stimulated intermittently with glutamate which triggered the release of the radioactively-labelled

neurotransmitter. Relative release of this neurotransmitter was quantified by measuring the level of radioactivity in fractions collected from the tissue run-off. The amount of radioactivity found in these fractions provided a proportional indication of how much radioactively-labelled neurotransmitter had been released. The *in-vitro* superfusion method described herein has been characterised in this laboratory over a number of years (Howells and Russell 2008, Warton, Howells et al. 2009, Sterley, Howells et al. 2013, Sterley, Howells et al. 2013, Sterley, Howells et al. 2014). Studies have included measuring release of tritiated norepinephrine ([^{3}H]NE) and tritiated dopamine ([^{3}H]DA) in several rat strains (e.g. spontaneous hypertensive rats, Wistar rats and S-D rats) and brain areas (e.g. hippocampus, prefrontal cortex and nucleus accumbens). Stimulation media have included glutamate, GABA and potassium as well as the incorporation of various other receptor agonists and antagonists. NE release is stimulated via the action of glutamate on AMPA and NMDA receptors in the hippocampus and prefrontal cortex (Howells and Russell 2008). Glutamate release is also known to be stimulated via the activation of adrenoceptors (Chen, Li et al. 2006). Additionally GABA serves to reduce the activity of NE neurons when applied to the LC (Szabo and Blier 2001). The relationship between these neurotransmitter systems is yet to be investigated within the context of the SIR model. One isolated and one socialised rat of the same sex and of the same age (i.e; from the same litter) were used in each superfusion experiment. This was to ensure the developmental stage was consistent between both brains. Superfusion experiments were carried out at 20-25 °C. On the day of testing each rat was weighed and rehoused in a clean individual cage and brought up to the laboratory for 60 minutes prior to experimentation in accordance with laboratory procedure. Three different superfusion protocols were used in this project to investigate the effects of social isolation rearing on neurochemistry under various conditions. Basic experimental set up for all superfusions was as follows.

Animals were killed by rapid decapitation with a guillotine between 09h00 and 10h00 so as to keep the time window similar to that used in the behavioural experiments. The brains were immediately and swiftly removed from skulls. First, scissors were used to cut through the skin between the ears and then rongeurs were used to carefully break away the superior surface of the skull, exposing the brain. Any remaining meninges were broken through using the end of a small spatula. The skull was then held at an angle, so that the brain could be easily scooped out, and dropped into ice-cold carbogenated Krebs slush buffer (118 mM NaCl, 4.7 mM KCl, 1.0 mM $NaH_2PO_4.H_2O$., 1.2 mM $MgCl_2.6H_2O$, 25 mM $NaHCO_3$, 11 mM D(+)-Glucose monohydrate, 1.3 mM $CaCl_2.H_2O$ (all salts from *Merck*) and 37 μM EDTA(*AnalR*)). The Krebs solution was bubbled with carbogen; 95 %

oxygen, 5 % carbon dioxide (*AirLiquide*) prior to the addition of the brain tissue in order for it to become saturated with the gases and to buffer the solution between pH 7.4-7.5.

After the tissue had bubbled in the ice-cold Krebs for 15 minutes the relevant brain areas were dissected out. The prefrontal cortex dissection involved first manually cutting 3 coronal slices from the most anterior part of the brain at 900 µm thickness (Figure 3.1) using a tissue chopper (*McIlwain);* this area represents the prefrontal cortex anterior to the genu of the corpus callosum. Any olfactory bulb tissue was discarded and the prefrontal cortex slices from both hemispheres were then sliced again with the tissue chopper at 300 µm thicknesses and then rotated 90° and chopped at 300 µm once more (Figure 3.2). Once the prefrontal cortex had been removed the hippocampus was dissected. A scalpel was used to score the superficial connections of the longitudinal fissure between the brain hemispheres. The cortex of each hemisphere was gently teased off using a small spatula to reveal the hippocampus (Figure 3.3). The hippocampus was then eased out using the spatula, taking care to ensure the deepest and most dorsal part was not lost. Any vasculature or myelination on the surface of the hippocampus was removed with tweezers. This was repeated for the other hippocampus and then both hippocampi were chopped perpendicularly into 300 µm x 300 µm columns using the tissue chopper (Figure 3.4).

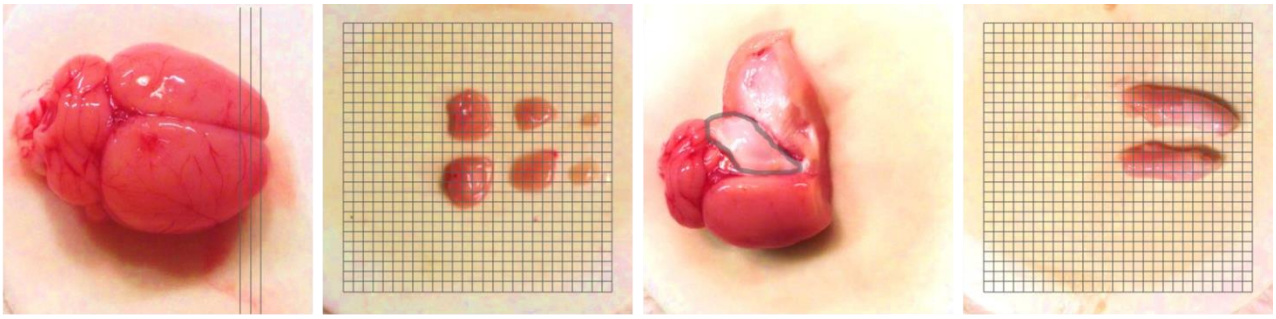

Figure 3- Tissue dissection

Tissue dissection of rat brain: 1. Whole rat brain and 900 µm prefrontal cortex slicing represented by grey lines. 2. Prefrontal cortex slices with orientation of 300 µm x 300 µm cross-cutting. 3. Cortex peeled back with hippocampus revealed and outlined in grey. 4. Hippocampi with orientation of 300 µm x 300 µm cross-cutting.

Cross-chopped tissue from each brain area was incubated in glass vials with 1 ml carbogenated ice-cold Krebs buffer containing 5.7 mM L-ascorbic acid (*Sigma Aldrich*), an antioxidant preventing the production of free radicals, in a water bath at 37 °C for 10 minutes. This was to allow for tissue to return to functional homeostatic temperature. From this stage onwards experiments were carried out under low intensity (non-fluorescent) lamp lighting so as not to cause excitation of the radioactivity. Radioactively-labelled neurotransmitter norepinephrine (2.67 µL DL-,[7-^{3}H(N)]-norepinephrine hydrochloride, 1 mCi/ml , 12 Ci/mmol *Perkin Elmer*) was added to each vial and incubated with brain tissue in the same water bath for a further 15 minutes to allow for uptake of the [^{3}H]NE into vesicles.

Excess solution was pipetted off so as to remove surplus radioactivity which had not been taken up into noradrenergic terminals. Tissue was then pipetted into the 16 superfusion columns on top of the cotton wool balls, brain areas were split evenly between their respective columns, (2 or 4 columns per brain area depending on experimental conditions, allowing for repeat data to be collected). Inlet tubes submerged in carbogenated Krebs buffer in the 37 °C water bath were connected to each column and solution was washed over tissue by means of a peristaltic pump at a speed of 0.25 ml / min. Tissue was first perfused with Krebs buffer for 80 minutes to remove any remaining radioactivity not taken up into the noradrenergic terminals. Following this, the motor of the fraction collector rack was engaged and every 5 minutes the rack was moved up so that the columns dripped into a new row of collecting vials, whilst being perfused with either plain Krebs buffer or a stimulating solution of Krebs buffer. Ten samples were collected per column, thus providing an indication of functional release of [^{3}H]NE from the tissue under differing conditions summarised in Table 4.

Once the first 9 samples had been collected the motor was disengaged and the cotton wool ball together with the tissue from each column was placed into the 10th sample vial with 1ml 0.1 M NaOH. Then 3.4 ml liquid scintillation fluid (Aquasafe 500plus *Zinsser Analytic*) was added to each sample vial to suspend radioactivity, thus permitting efficient counting of the radioactivity by optimising photon emission. Radioactivity in degradations per minute (DPM) were recorded by a Packard 1900 TRI-CARB liquid scintillation analyser based on a standard decay curve generated from quenched tritium standard samples (Figure 86). Fractional release of [^{3}H]NE was calculated from the DPMs for each column of tissue. First the DPMs from each 5 minute fraction were divided by the total amount of radioactivity in the tissue at the time of release of that fraction (the sum of the fraction in question added to all subsequent fractions). This provided the fractional release of [^{3}H]NE relative to the total radioactivity at the time the fraction was collected. Then, the

fractional release value calculated for the preceding baseline fraction was subtracted from each stimulation fraction value and multiplied by 100 to give percentage release of [^{3}H]NE for each stimulation.

An average was calculated for each of the three stimulations for all columns containing identical tissue receiving identical stimulation protocol (Figure 4). Column data were removed from the analysis where the first stimulation was lower than the initial baselines, accepted as error within the experimental set-up for that column only.

Table 4- SF1, 2, 3 collection vial order and stimulation sequences

Order of superfusion solution contents for protocols 1, 2, and 3. Each row represents a 5 minute fraction where HC = hippocampus, PFC = prefrontal cortex, Glu = glutamate, Ant = ionotropic glutamate antagonist; CNQX or MK-801.

Vial number	Vial description	**Superfusion 1** HC & PFC	**Superfusion 2** HC	**Superfusion 3** HC & PFC
.1	Baseline 1	Krebs	Krebs	Krebs
.2	Baseline 2	Krebs	Krebs	Krebs
.3	Stimulation 1	1 min Glu-Krebs 4 min Krebs	1 min Glu/Ant-Krebs 4 min Krebs	1 min Glu/GABA-Krebs 4 min Krebs
.4	Baseline 3	Krebs	Krebs	Krebs
.5	Stimulation 2	1 min Glu-Krebs 4 min Krebs	1 min Glu/Ant-Krebs 4 min Krebs	1 min GABA/Glu-Krebs 4 min Krebs
.6	Baseline 4	Krebs	Krebs	Krebs
.7	Stimulation 3	1 min Glu-Krebs 4 min Krebs	1 min Glu/Ant-Krebs 4 min Krebs	1 min high KCl-Krebs 4 min Krebs
.8	Baseline 5	Krebs	Krebs	Krebs
.9	Drying	Remove inlet tubes from solution, columns run dry	Remove inlet tubes from solution, columns run dry	Remove inlet tubes from solution, columns run dry
.10	Pellet	Tissue from column on cotton wool ball with 1ml 0.1M NaOH	Tissue from column on cotton wool ball with 1ml 0.1M NaOH	Tissue from column on cotton wool ball with 1ml 0.1M NaOH

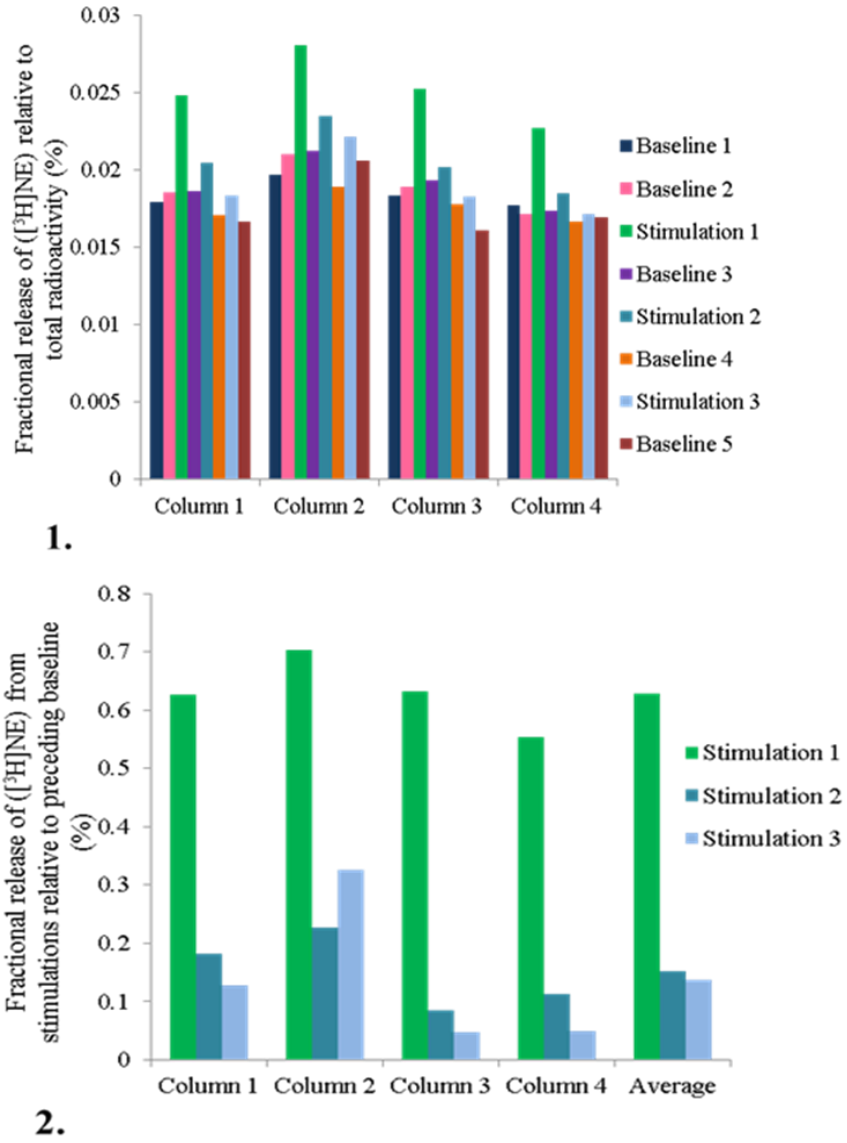

Figure 4- Superfusion fractional release, example of calculation

Representative graphs from a superfusion experiment. Graph 1 shows fractional release of [^{3}H]NE from each 5 minute fraction as a percentage of the total radioactivity present in the tissue at the time of release in four repeat columns (containing the same brain area undergoing the same stimulation protocol). Graph 2 shows percentage release of [^{3}H]NE from stimulations 1,2 and 3 calculated from stimulation peak and preceding baseline values in graph 1. For each corresponding column of tissue; the baseline 2 value was subtracted from the stimulation 1 value and multiplied by 100, the baseline 3 was subtracted from the stimulation 2 value and multiplied by 100, and the baseline 4 was subtracted from the stimulation 3 value and multiplied by 100. An average for each stimulation was then calculated from the four columns for each brain area and stimulation protocol.

2.3.1 SF 1- GLUTAMATE-STIMULATED [3H]NE RELEASE IN HIPPOCAMPUS AND PREFRONTAL CORTEX

The first group of superfusion experiments measured relative release of [^{3}H]NE from prefrontal cortex and hippocampus when stimulated with glutamate-containing Krebs buffer FS n=12, FI n=12, MS n=10 and MI n=10. Stimulations 1, 2 and 3 consisted of superfusion with Krebs buffer containing 250 µM L-Glutamic Acid (*Sigma Aldrich*). The purpose of these experiments was to probe how NE release might differ between sex and housing groups upon excitation with glutamate. This concentration was previously determined in our laboratory as sufficient to elicit [^{3}H]NE release in superfusion experiments (Howells and Russell 2008, Mc Fie, Sterley et al. 2012). During these stimulations all inlet tubes were moved from the plain Krebs buffer into the glutamate-containing Krebs buffer for the first 1 minute then back to the plain Krebs buffer for the remaining 4 minutes of the fraction (Table 4), (Table 5). The first 7 superfusion experiments were completed and analysed in the beta counter, the samples were then disposed of due to limited radioactive storage space. Subsequently it was noted that the beta counter was not recording DPMs consistently. Alterations were made so that the room in which the counting took place was completely dark and the vials for counting were of optimal size. For this reason the first 7 experiments were excluded from the final analysis. This meant that the total number of animals whose data were analysed was FS n=7, FI n=7, MS n=8 and MI n=8.

Table 5- SF1, column order

Row A- Column number

Row B- Brain tissue (HC = hippocampus, PFC= prefrontal cortex)

Row C- Animal housing conditions (Iso = isolated, Soc = socialised).

A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B	HC	HC	HC	HC	PFC	PFC	PFC	PFC	HC	HC	HC	HC	PFC	PFC	PFC	PFC
C	Iso	Iso	Iso	Iso	Iso	Iso	Iso	Iso	Soc	Soc	Soc	Soc	Soc	Soc	Soc	Soc

2.3.2 SF 2- GLUTAMATE-STIMULATED [3H]NE RELEASE IN HIPPOCAMPUS IN PRESENCE OF MK-801 AND/OR CNQX

The second group of superfusion experiments measured relative release of [^{3}H]NE in the hippocampus only, when stimulated by glutamate in the presence of ionotropic glutamate AMPAR and NMDAR antagonists CNQX (6-cyano-7-nitroquinoxaline-2, 3-dione) and MK-801 (Dizocilpine) respectively FS n=7, FI n=7, MS n=7 and MI n=7. This was to provide information on the functionality of these glutamate receptors and their contributions to NE function. Stimulations 1, 2 and 3 consisted of superfusion with glutamate-containing Krebs buffer (same as in superfusion 1 protocol) with the addition of either 1) nothing as a control condition, 2) 1 µM CNQX, 3) 10 µM MK-801 or 4) 1 µM CNQX and 10 µM MK-801 in combination (Howells and Russell 2008). During these stimulations all inlet tubes were moved from the plain Krebs buffer into one of the four abovementioned Krebs buffer solutions for the first 1 minute then back to the plain Krebs buffer for the remaining 4 minutes of the fraction (Table 4, Table 6). Two superfusion experiments were excluded because the data showed that there was no effect of stimulation FS n=6, FI n=6, MS n=6 and MI n=6. This was likely due to a blockage of the inlet tube; inlet tubes were routinely replaced when blockages were detected. Only one brain area could be assayed in this part of the study due to the number of columns available for repeats. The hippocampus was chosen over the prefrontal cortex as in testing it was found that relatively more [^{3}H]NE was released in the hippocampus upon stimulation with glutamate.

Table 6- SF2, column order

Row A- Column number

Row B- Brain tissue (HC = hippocampus)

Row C- Animal housing conditions (Iso = isolated, Soc = socialised)

Row D- Krebs buffer solution composition (G = glutamate, C = CNQX, M = MK-801)

A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B	HC	HC	HC	HC	HC	HC	HC	HC	HC	HC	HC	HC	HC	HC	HC	HC
C	Iso	Iso	Iso	Iso	Iso	Iso	Iso	Iso	Soc	Soc	Soc	Soc	Soc	Soc	Soc	Soc
D	G	G	G,C	G,C	G,M	G,M	G,C,M	G,C,M	G	G	G,C	G,C	G,M	G.M	G,C,M	G,C,M

2.3.3 SF 3- GLUTAMATE, GABA AND KCL-STIMULATED [3H]NE RELEASE IN HIPPOCAMPUS AND PREFRONTAL CORTEX

The third group of superfusion experiments measured relative release of [^{3}H]NE in the prefrontal cortex and hippocampus when tissue was stimulated first by Krebs buffer containing glutamate and then γ-aminobutyric acid (GABA) or vice versa and then for the third stimulation with Krebs buffer containing a high concentration of KCl, FS n=18, FI n=18, MS n=7 and MI n=7. The purpose of these experiments was to assess the role of excitatory and inhibitory neurotransmitters on NE activity. Glutamate-containing Krebs buffer was prepared by the same method as stated in the superfusion 1 protocol. Other stimulating Krebs buffer solutions were; 100 µM GABA (*Sigma Aldrich*)-containing Krebs buffer and high concentration KCl-containing Krebs buffer (98 mM NaCl, 25 mM KCl, 1.0 mM $NaH_2PO_4.H_2O$., 1.2 mM $MgCl_2.6H_2O$, 25 mM $NaHCO_3$, 11 mM D(+)-Glucose monohydrate, 1.3 mM $CaCl_2.H_2O$), (all salts from *Merck*) and 37 µM EDTA (*AnalR*). These concentration had been determined by previous work in our laboratory (Sterley, Howells et al. 2013). The volumes of the salts were adjusted accordingly to achieve these different molarities. The high concentration KCl-containing Krebs buffer was used to evoke depolarization-induced release of neurotransmitter as an indicator of neurotransmitter reserves. During baseline fraction collections, all inlet tubes were submerged in Krebs buffer and during stimulations, inlet tubes were moved into glutamate/GABA/high concentration KCl-containing Krebs buffer for the first 1 minute then back to the plain Krebs buffer for the remaining 4 minutes of fraction collection. During the first stimulation half of the inlet tubes were moved from plain Krebs buffer into glutamate-containing Krebs buffer and the other half were moved into GABA-containing Krebs buffer. For the second stimulation inlet tubes were moved into whichever glutamate-containing or GABA-containing Krebs buffer they had not been in for the previous stimulation. For the third and final stimulation all inlet tubes were moved into high concentration KCl-containing Krebs buffer, (Table 4, Table 7). Once experiments not showing effective stimulations had been removed the total number of animals was as follows FS n=12, FI n=12, MS n=6 and MI n=6.

Table 7- SF3, column order

Row A- Column number

Row B- Brain tissue (HC = hippocampus, PFC = prefrontal cortex)

Row C- Animal housing conditions (ISO = isolated, SOC = socialised)

Row D- First stimulation Krebs buffer solution composition (G = glutamate, GA = GABA)

Row E- Second stimulation Krebs buffer solution composition (G = glutamate, GA = GABA)

Row F- Third stimulation Krebs buffer solution composition (K = high concentration KCl)

A	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
B	HC	HC	HC	HC	PFC	PFC	PFC	PFC	HC	HC	HC	HC	PFC	PFC	PFC	PFC
C	Iso	Iso	Iso	Iso	Iso	Iso	Iso	Iso	Soc	Soc	Soc	Soc	Soc	Soc	Soc	Soc
D	G	G	GA	GA	G	G	GA	GA	G	G	GA	GA	G	G	GA	GA
E	GA	GA	G	G	GA	GA	G	G	GA	GA	G	G	GA	GA	G	G
F	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K	K

2.4 ELISA DETERMINATION OF NE AND GLUTAMATE CONCENTRATIONS IN HIPPOCAMPUS AND PREFRONTAL CORTEX

Competitive enzyme-linked immunosorbent assay (ELISA) kits were used for quantitative determination of NE and glutamate in tissue homogenates of prefrontal cortex and hippocampus tissue *(LDN immunoassays and services: Noradrenaline Research ELISA, Glutamate ELISA*). For full instructions refer to appendix (Figure 93, Figure 94, Figure 98 and Figure 99).

The principle of a competitive ELISA is to measure endogenous concentrations of peptides/proteins/antibodies/hormones. In the present study concentrations of glutamate and NE were investigated using ELISAs. Antigens to the specific neurotransmitter (antibody) are bound in solid state to the wells of the ELISA plate. Thus, when brain tissue homogenate is added, the antigen pre-coating on the plate will compete for binding sites, i.e. the respective neurotransmitter bind to their respective antigen. Then, any antigen-antibody complexes not bound to the solid phase will be washed away. Following this a secondary antibody is added which binds to the solid phase antigen-antibody complexes left in the wells. The secondary antibody is conjugated to a molecule which can be detected using a chromogenic substrate when read at 450 nm. In a competitive assay the mean optical densities calculated will be inversely proportional to the analyte concentration.

Therefore a low signal detected in the solid-phase antigen-antibody complexes indicates a high level of sample/control/standard antigen-antibody complexes have been washed off, thus indicating a high level of the antigen of interest has been detected.

In previous studies NE levels were found to fluctuate around 200 ng/g (concentration NE / wet weight tissue) in the hippocampus and cortex of S-D rats when determined by HPLC (Jiang, Li et al. 2014). These rats had also been subject to sodium taurocholate injections in the bile-pancreatic duct, however this was found not to affect the neurotransmitters assayed in brain tissue and was therefore a suitable starting point when choosing the concentrations to use in this study. Another group using HPLC determined levels of NE in the hippocampus and prefrontal cortex at 233 and 252 ng/g respectively in control groups of Wistar rats (Del Pino, Martínez et al. 2011). HPLC has also been used to measure glutamate in the hippocampus and prefrontal cortex in S-D rats with concentrations found to be 6.5 and 11.2 mg/g respectively (Liu, Tang et al. 2011).

Four ELISAs were performed in order to quantify NE in the hippocampus, NE in the prefrontal cortex, glutamate in the hippocampus and glutamate in the prefrontal cortex. Animals were brought up to the laboratory 60 minutes prior to experimentation. Each animal was rapidly decapitated using a guillotine and the head was immediately and briefly submerged in liquid nitrogen in order to reduce deterioration of the tissue during dissection. The brain was removed by the same procedure as stated in the superfusion set up. The most anterior portion of the frontal cortex (5mm) was sliced off coronally using a scalpel blade (Figure 3.1). Both hippocampi were dissected out whole using the same technique as detailed in the superfusion set up, without cross chopping (Figure 3.3, Figure 3.4). Each brain area was placed in its entirety into a cryo-vial, snap frozen and stored in liquid nitrogen until such time as they were used in the assay.

ELISAs were carried out at 20-25 °C. On the day of the experiment the tissue was thawed on ice. The tissue from the hippocampus and prefrontal cortex of each animal were weighed. Sample buffer was added according to the weight of the tissue so that homogenates of 200 mg/ml could be made for each sample. The sample buffer contained 1 mM EDTA (AnalR) and 4mM sodium metabisulfate (*Merck*) and 0.01 N HCl (*Merck*) to prevent catecholamine degradation and to provide optimal pH and solubility. The tissue was homogenised in buffer by sonication for 10-15 seconds so that sound waves could break up tissue membrane and vesicular compartments to allow the neurotransmitters of interest in all parts of the cell to bind to the kit antigens.

A preliminary concentration optimisation assay was performed for each of the two ELISA kits. One tissue homogenate from each brain area was diluted to a number of concentrations ranging from 1

mg/ml to 200 mg/ml. This range was chosen based on previous studies and detailed above. ELISAs were carried out as per the manufacturer's instruction (detailed below). The different concentrations were assayed against standard and control samples supplied with the kit. This was to ascertain which concentrations would provide an optimal reading on the linear section of the standard spline curve generated. Optimal concentrations chosen based on this first determination were 5 mg/ml and 10 mg/ml to ensure that data were readable on the graph for both NE and glutamate experiments (Figure 95, Figure 100). Two different concentrations were chosen due to slight variability between the hippocampus and prefrontal cortex

Once the optimal concentrations had been decided a separate ELISA was carried out on all samples for each neurotransmitter, brain area combination. Sample sizes were as follows Hippocampus NE: FS n=6, FI n=6, MS n=6 and MI n=6, prefrontal cortex NE: FS n=6, FI n=6, MS n=6 and MI n=6, hippocampus glutamate: FS n=10, FI n=10, MS n=10 and MI n=10 and prefrontal cortex glutamate FS n=6, FI n=6, MS n=6 and MI n=6. For each ELISA 5 mg/ml and 10 mg/ml concentration were prepared for each sample by diluting thawed homogenates with sample buffer. These were used as repeats.

For the NE ELISAs the extraction plate phase was performed first. This involved pipetting 10 µl of the standards, controls, and samples into each well and then adding a further 90 µl distilled water and 25 µl of TRIS-EDTA buffer to each well. Importantly, each homogenate was vortexed for 10 seconds immediately before being added to the plate. This ensured that the heavier tissue had not separated from the buffer. The plate was placed in a plate shaker for 60 minutes at 600 revolutions per minute (rpm). Following this the plate was inverted to remove all liquid. Next, 1 ml wash buffer was added to each well and it was returned to the shaker for 5 minutes, the plate was then inverted and this step was repeated. Acylation of the plates involved adding 150 µl acylation buffer and 25 µl acylation reagent to the wells and shaking for 20 minutes then inverting the plate. The plate was then washed twice more with wash buffer as previously described. Following this 100 µl HCl was added to each well and the plate was shaken for 10 minutes. These steps optimise the pH of the supernatant for the subsequent enzymatic conversion.

The following steps involved the microtiter plate. From the extraction plate 90 µl of the supernatant was pipetted in to the microtiter plate making sure to maintain the same sample order between plates. Then 25 µl of a pre-prepared enzyme solution was added to the wells and the plate was shaken for 1 minute then incubated for two hours at 37 °C.

The final stages were carried out in the NE microtiter strips. The NE microtiter strips were pre-coated with an NE antigen so that the anti-NE antibody would bind to the plate. From the microtiter plate 100 µl from each well was pipetted into the NE microtiter strips plate. Then 50 µl of NE antiserum was added to every well and the plate was shaken for 1 minute. This was a rabbit anti-NE antibody which bound to NE in the sample and on the plate. NE in the sample competed with NE on the microtiter strips plate. The plate was then incubated in the fridge (4 °C) overnight to ensure maximal binding site occupation. In the morning the plate was inverted and was washed 4 times by adding 300 µl wash buffer to each well and then inverting. This removed any anti-NE antibody that was not bound to NE on the plate. Next, 100 µl of the enzyme conjugate was added and the plate was shaken for 30 mins. This was a goat anti-rabbit immunoglobulin which would bind to the rabbit antibody. The 4-step wash procedure was then repeated. Next, 100 µl of the chromogenic substrate was added to all wells and the plate was shaken for 25 minutes. This step tagged the NE bound to the plate with a chromogenic molecule which could be detected in the plate reader at 450 nm. Lamp lighting was used from this phase onwards so as not to excite the substrate. Finally 100 µl stop solution was added to end any reactions and the plate was read at a wavelength of 450 nm.

The principle of the glutamate ELISAs was the same as for the NE ELISA and the procedures were largely the same. Briefly, 100 µl of the samples, standards and controls were added to the extraction plate along with 100 µl of diluent and the plate was shaken for 10 minutes. Then 25 µl from each well of the extraction plate was pipetted into the wells of the reaction plate. An additional 10 µl NaOH, 50 µl equalising reagent and 10 µl of D-reagent were then added to each well and the plate was shaken for two hours. Then 75 µl Q-buffer was added to each well and the plate was shaken for a further 190 minutes.

For the ELISA itself, 25 µl from each extraction plate well was pipetted into the glutamate microtiter strips plate and 50 µl glutamate antiserum was added. The plate was then incubated in the fridge (4 °C) overnight to ensure maximal binding site occupation. The plate was washed and inverted 3 times using 300 µl wash buffer and then 100 µl of the enzyme conjugate was added to each well and the plate was shaken for 30 mins. The 3-step washing procedure was then repeated, ensuring no unbound glutamate antibody remained in the wells. Next 100 µl of the chromogenic substrate was added to all wells and the plate was shaken for 25 minutes, again, lamp lighting was used from this step onwards. Finally 100 µl stop solution was added to end any reactions and the plate was read at a wavelength of 450 nm.

The plate reader gave the optical density value of each well. These optical density values were subtracted from an average of the background values (the first standard with no neurotransmitter at all). These values were entered into the online ELISA calculation tool elisaanalysis.com for each of the four brain area and neurotransmitter combinations. Standard curves were generated and the corresponding concentrations of each sample were calculated according to this standard curve (Figure 96, Figure 97, Figure 101 and Figure 102). The regression algorithm used was a 4-paramter logistic regression. The resulting neurotransmitter concentrations from the original 5 mg/ml and 10 mg/ml (tissue per volume of buffer) samples were divided by 5 and 10 respectively and an average was taken of the two values. The final concentrations were expressed as neurotransmitter per wet weight of tissue.

2.4.1 BCA PROTEIN ASSAY

The bicinchoninic acid protein assay (*Pierce™ BCA Protein Assay Kit*) is used to determine the total concentration of protein in a solution via colorimetric analysis of a reduction of copper ions (Cu^{2+} - Cu^{1+}) (Figure 103, Figure 104). This reduction is known as the biuret reaction and takes place due to the presence of protein in an alkaline environment (Smith, Krohn et al. 1985). One the reduction has taken place the Cu^{1+} chelates with the BCA leading to a measureable colour change. The BCA assay was used as a follow-up to the ELISA so that neurotransmitter concentrations could be expressed per wet weight of tissue and also per total protein. Frozen tissue in sample buffer from ELISAs at 200 mg/ml concentrations was thawed and made up to 5 mg/ml concentrations in RIPA (Radio immunoprecipitation assay) buffer containing 150 mM NaCl (Merck), 1 % Triton-X 100 (Merck), 0.1 % Sodium Dodecyl Sulfate (Sigma), 20 mM Tris (hydroxymethyl) aminomethane (pH7.5) (Merck), 1 % Sodium Deoxycholate (Sigma). This assay was performed on lysates from hippocampus-NE, prefrontal cortex-NE and hippocampus-glutamate ELISA experiments. Unfortunately homogenates from the first day of testing (prefrontal cortex-glutamate ELISA) were not frozen immediately so were not suitable for further BCA tests. BCA assays were performed following microplate procedure. First 25 µl of each standard and sample were pipetted into the wells and 200 µl of working reagent was added then the plates were placed on a plate shaker for 30 seconds then incubated at 37 °C for 30 minutes. Finally, absorbances were measured on a plate reader set to 562nm. Standard curves were made and the corresponding concentrations of each sample were calculated according to this standard curve

(Figure 105, Figure 106 and Figure 107). Concentrations from the BCA assay were converted into the same units as ELISA values, in order to express results as neurotransmitter per total protein ELISA concentrations were divided by BCA concentrations.

2.5 ULTRASONIC VOCALISATION TESTING

The aim of this part of the study was to provide insight into the impact of social isolation on communicative behaviour to address negative symptomatology of schizophrenia by analysing ultrasonic vocalisation recordings from each animal. Rats emit vocalisations at ultrasonic frequencies; these vocalisations have a communicative function in social context and also indicate affective state (Portfors 2007). Calls in the 22 kHz frequency band with durations between 300 ms-3000 ms provide an indication of fear, anxiety or negative affect and are present in aversive environments (Litvin, Blanchard et al. 2007, Ouda, Jílek et al. 2016). Studies from socially isolated rats have typically shown that isolated animals emit significantly fewer calls in the 300ms – 3000ms 22 kHz range than their socialised counterparts (Nunes Mamede Rosa, Nobre et al. 2005, Inagaki and Mori 2013). This reduction in calls can be interpreted as an indication of abnormal communication in isolated animals and social withdrawal.

In the present study recordings were taken in the 22 kHz range. During testing one isolated animal and one socialised animal of same sex and age were each recorded from using two separate bat detectors (Figure 5) FS n=16, FI n=16, MS n=8 and MI n=8. The protocol was replicated from previous studies by our lab (Dimatelis, Vermeulen et al. 2016). One animal was placed in each of the two transparent Plexiglas cylinders (height 70 cm, diameter 28 cm), 3 cm apart so that during the experiment the animals could see each other, thus providing stimulus for communication, no other rats were present in the room at the time of recording. Each cylinder was cleaned with 70 % ethanol and then water after every experiment and lined with fresh sawdust, as hormonal scent cues have been shown to impact calling behaviour, in addition to this, only one sex of rat was tested per day (Geyer and Barfield 1978). A directionally sensitive bat detector (Mini-3, Ultra Sound Advice) was hung halfway down each cylinder, aimed downwards towards the cylinder floor and the rat being tested, so that it detected only the vocal emissions from the animal directly below it and not from the rat in the adjacent cylinder. These recordings were processed through UltraVox software (Noldus Information Technology, Wageningen, Netherlands). Vocalisations provided an indicator

of response to a novel environment given that it was the first time animals were not in their home cage. Animals were gently placed in Plexiglas cylinders and a 10 minute recording was initiated by the experimenter who then exited the behavioural suite. Software recorded call duration (ms) at 22 kHz range. Call durations of 300 ms- 3000 ms were analysed and shorter or longer lengths removed from analysis, as these call durations at 22 kHz are associated with anxiety (Anderson 1954, Brudzynski 2005, Portfors 2007, Inagaki and Mori 2013). Mean call durations and total number of calls were the two parameters statistically analysed. An initial malfunction of the equipment meant that data from 7 animals had to be removed from the analysis, this malfunction was addressed and recordings for all other animals were made FS n=14, FI n=16, MS n=5 and MI n=6. Following this, it was noted that the two bat detectors seemed to be recording with different sensitivities (i.e.: Channel A seemed to be making consistently more recordings than Channel B). To ensure that this did not bias the data, socialised and isolated animals were alternated between the two different recorders and chambers. This difference in the recording equipment was analysed and corrected for by applying a scale factor so that results obtained from the detectors were comparable (Appendix: A.5). Following behavioural experiments all rats were returned to the animal facility and housed as before.

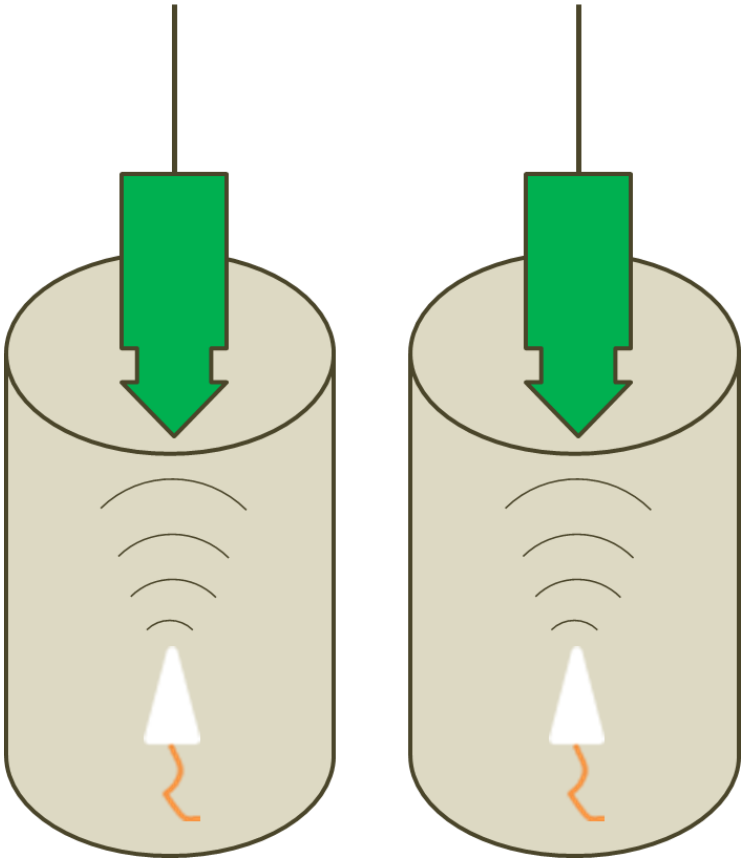

Figure 5- Ultrasonic vocalisation test apparatus

During each experiment, two separate recordings were made of ultrasonic vocalisations from one socialised and one isolated rat at the 22 kHz range. One rat from each housing condition was placed into each of the transparent Plexiglas cylinders. Recordings of the calls from each rat were made by a directionally-sensitive bat detector hanging halfway down each cylinder.

2.6 STATISTICAL ANALYSES

All statistical analyses were carried out using Dell Statistica data analysis software system, version 13. Dell Inc. (2015). All graphs were designed with Microsoft Excel (2010). Data were tested firstly for main housing effects * between socialised and isolated animals within sex groups (i.e.: I vs. S for parametric tests or MS vs. MI / FS vs. FI for nonparametric tests), then for sex differences # (i.e.: M vs. F for parametric tests or MS vs. FS / MI vs. FI for nonparametric tests) and then any experimental differences ^ (Channel A vs. B / Quadrant 1 vs. 2 etc.). Comparisons across housing and sex (i.e.: MS vs. FI / MI vs. FS) are included in appendices but not results section. Each data set was tested for normal distribution using Shapiro-Wilk W test where $p>0.05$ met criterion for normal distribution. If the data set were of normal distribution parametric factorial or repeated measures analysis of variance (ANOVA) was performed with sex (male, female) and housing (isolated, socialised) as categorical variables followed by Bonferroni post-hoc testing of significant effects. Parametric data are reported with their mean and standard deviation (SD).

If the data set did not fit the criterion for normal distribution and easily converted to a normal distribution using standard data transform functions then an appropriate nonparametric analysis was selected; Kruskal Wallis test, Friedman test, Wilcoxon test or Mann Whitney U test (with correction for multiple comparisons where appropriate). For nonparametric analyses, grouping variables were flattened into sex_housing, to create groups; female_isolated (FI), female_socialised (FS), male_isolated (MI) and male_socialised (MS). Nonparametric data are reported with their median, interquartile range (IQR), minimum (min) and maximum (max) values.

CHAPTER 3
-RESULTS-

3.1 BODY WEIGHT

Statistical tests were applied to body weight data (Figure 6) (n=175, 50 FS, 49 FI, 38 MS, 38 MI). At p42-49 differences were found between the groups ($H_{(3, N=175)}$ =30.27 p<0.0001), comprising of a main effect of housing where MI was heavier than MS, p=0.0487, and sex differences where MI was heavier than FI, p=0.0078, and MS was heavier than FS, p=0.046. At p49-56 differences were found between the groups ($H_{(3, N=175)}$ =58.99 p<0.0001), comprising of sex differences where MI was heavier than FI, p<0.0001 and MS was heavier than FS, p<0.0001. For all of the following weeks differences were found between the groups; p56-63 ($H_{(3, N=175)}$ =109.6 p<0.0001), p63-70 ($H_{(3, N=175)}$ =125.5 p<0.0001), p70-77 ($H_{(3, N=175)}$ =129.1 p<0.0001), p77-84 ($H_{(3, N=175)}$ =130.8 p<0.0001) and p84-91 ($H_{(3, N=175)}$ =131.4 p<0.0001). These results showed sex differences such that MS was heavier than FS and MI was heavier than FI, p<0.0001, for all results. Full statistical tables can be found in appendix A.1.

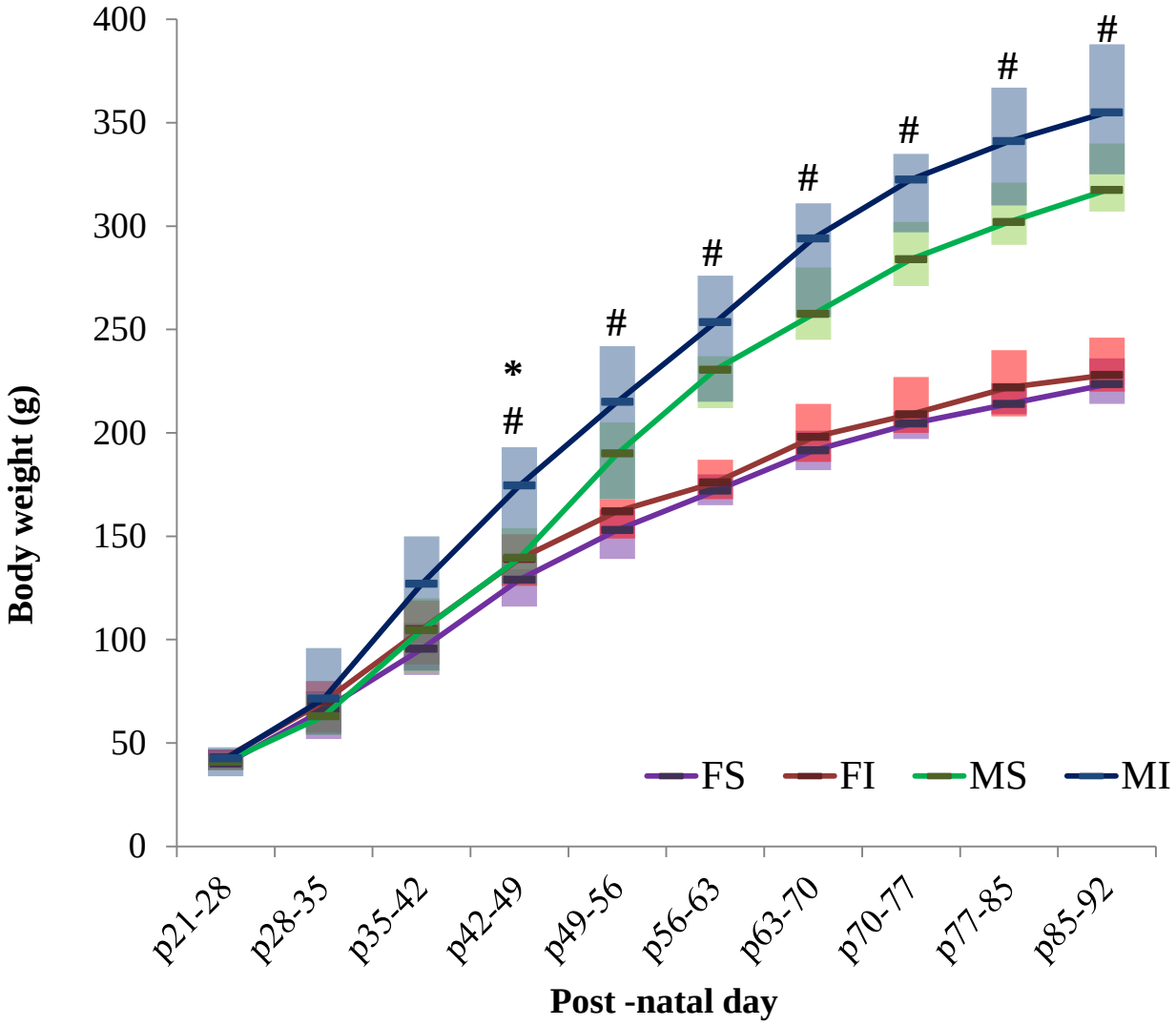

Figure 6- Body weight

Body weight recorded weekly from post-natal day 21 through to post-natal day 92 for each sex_housing group; female socialised (FS n=50) and female isolated (FI n=49), male socialised (MS n=38), male isolated (MI n= 38). Differences are indicated as follows: *Main effect of housing (MI vs. MS), # Sex difference (MS vs. FS / MI vs. FI), where first group listed in pair was heavier. Differences FS vs. MI, FI vs. MS not shown. Data are presented as median ± IQR, see text for individual p values.

3.2 NOVEL OBJECT RECOGNITION TEST

3.2.1 PHASE 1- OPEN FIELD EXPLORATION ANALYSIS

Three temporal analyses were performed on the phase 1 data including; the first minute, the first five minutes and the full ten minutes (Figure 7).

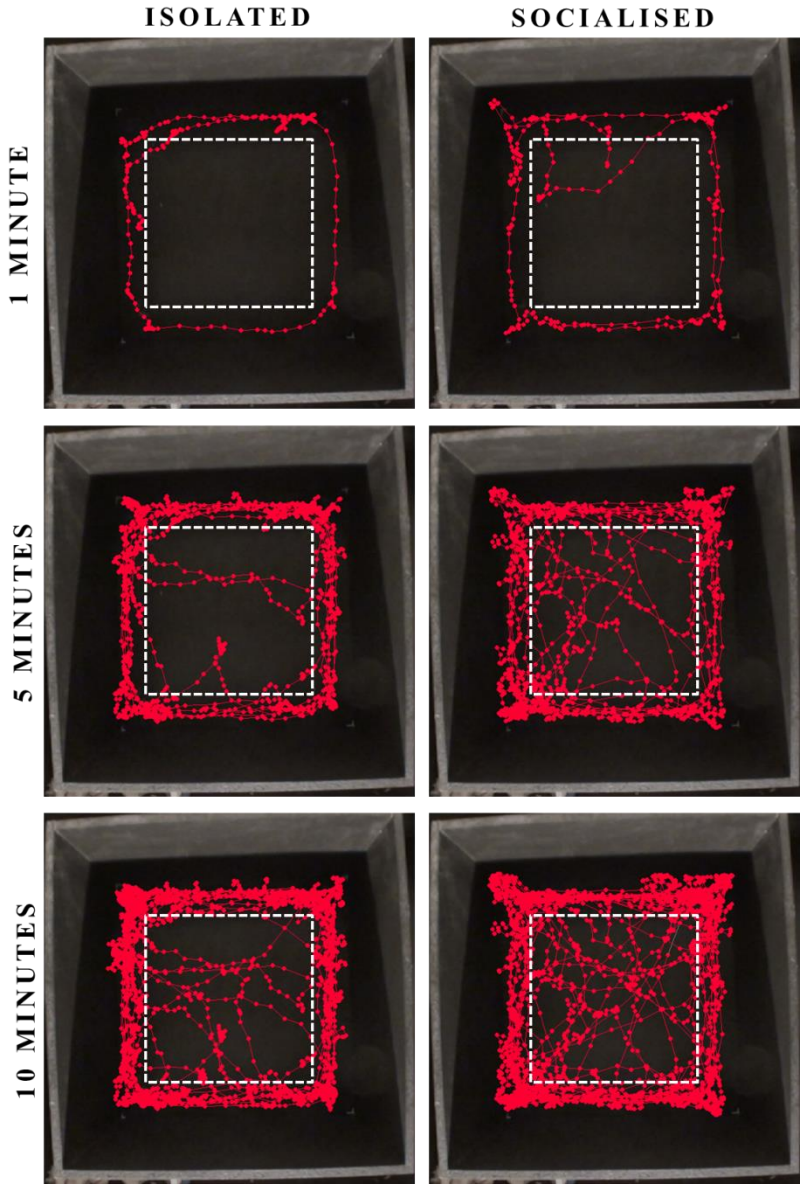

Figure 7- NOR P.1, Example traces

Sample analyses showing the cumulative exploration of two rats over the time course of the open field test as tracked with centre-point detection by *Noldus* software.

3.2.1.1 P.1- FIRST MINUTE

Data from the first minute of the open field exploration trial (phase 1) were analysed, (n=97, 24 FS, 23 FI, 25 MS, 25 MI). All phase 1 variables were tracked using centre-point detection (Figure 2.1). The following parameters were analysed; distance travelled in the arena (cm), time spent within the inner-zone (s) and number of entries into the inner-zone. Full statistical tables can be found in appendix A.2.1.1.

For the distance travelled (cm) (Figure 8), a main effect of housing was found ($F_{(1, 93)}$ =7.363, p=0.0079), where socialised animals travelled further than isolated animals, p=0.0080. Sex differences ($F_{(1, 93)}$ =10.82, p=0.0014), were also found where females travelled further than males, p=0.0012. Neither housing nor sex differences were found for the time spent in the inner-zone (s) (Figure 9). For the number of entries made into the inner-zone (Figure 10), a sex difference was found ($H_{(3, N=97)}$ =17.00, p=0.0007), where socialised males made more entries into the inner-zone than socialised females, p=0.0208, and isolated males made more entries into the inner-zone than isolated females, p=0.0387.

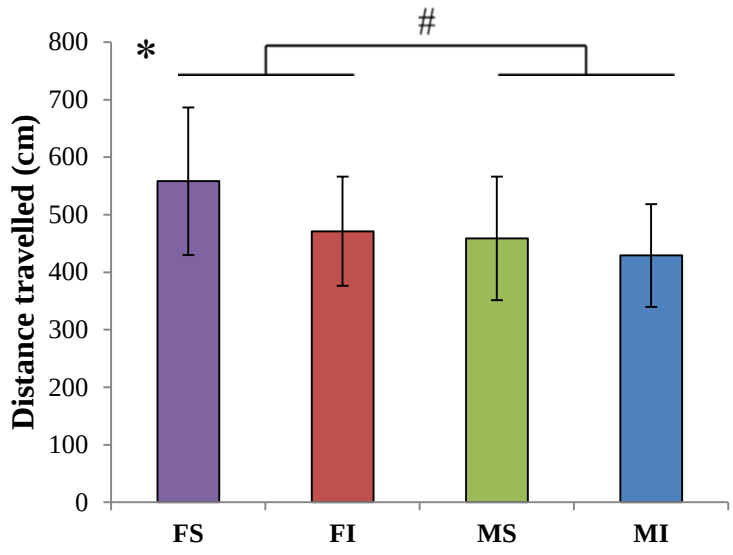

Figure 8- NOR P.1 1 MIN, Distance travelled (cm)

Distance travelled (cm) during open field exploration (phase 1) for each sex and housing group; female socialised (FS n=24), female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=25). *A main effect of housing was found, p=0.0079, where socialised animals

travelled further than isolated animals, p=0.0080. #A sex difference was found, p=0.0014, where females travelled further than males (p=0.0012). Data are presented as mean ± SD.

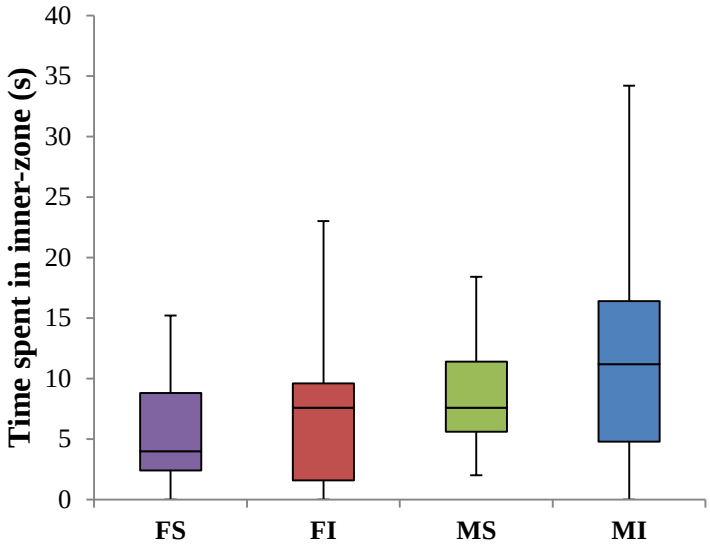

Figure 9- NOR P.1 1 MIN, Time spent in the inner-zone (s)

Time spent in the inner-zone (s) during open field exploration (phase 1) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=25). Neither housing nor sex differences were found. Data are presented as median ± IQR with min and max values

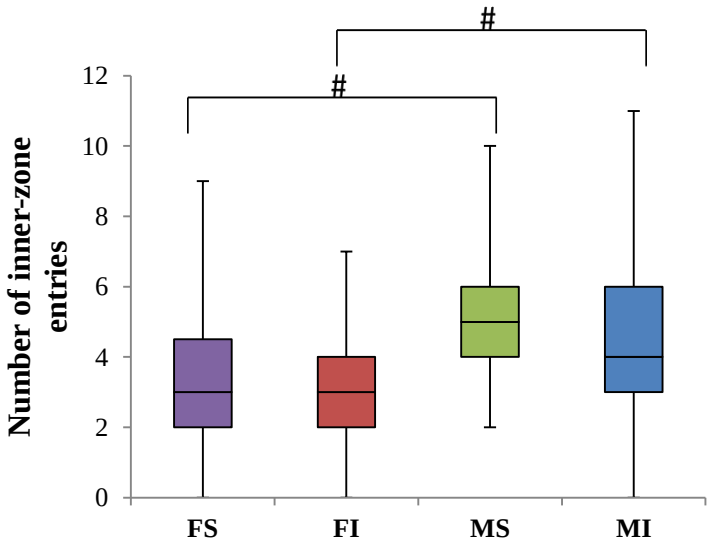

Figure 10- NOR P.1 1 MIN, Number of inner-zone entries

Number of entries into the inner-zone during open field exploration (phase 1) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and

male isolated (MI n=25). #A sex difference was found, p=0.0007, where MI were found to enter the inner-zone more frequently than FI, p=0.0387, and MS were found to enter the inner-zone more frequently than FS, p=0.0208. Data are presented as median ± IQR with min and max values.

3.2.1.2 P.1- FIVE MINUTES

Data from the first five minutes of the open field exploration trial (phase 1) were combined and analysed, (n=96, 24 FS, 23 FI, 25 MS, 24 MI). All variables from phase 1 were tracked using centre-point detection (Figure 2.1). The following parameters were analysed; distance travelled in the arena (cm), time spent within the inner-zone (s) and number of entries into the inner-zone. Full statistical tables can be found in appendix A.2.1.2.

Neither housing nor sex differences were found for distance travelled (cm) (Figure 11). Neither housing nor sex differences were found for the time spent (s) in the inner-zone (Figure 12). For the number of entries made into the inner-zone (Figure 13), a sex difference was found ($F_{(1, 92)} = 8.207$, p=0.0052), where males made more entries into the inner-zone than females, p=0.0055.

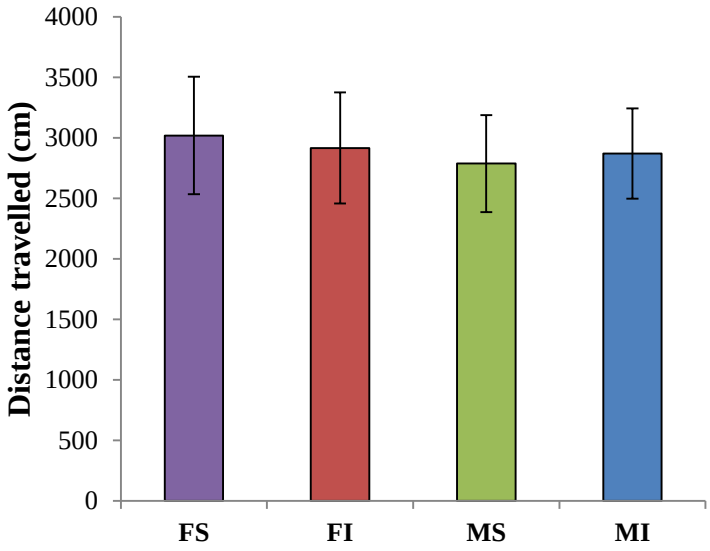

Figure 11- NOR P.1 5 MIN, Distance travelled (cm)

Distance travelled (cm) during open field exploration (phase 1) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male

isolated (MI n=24). Neither housing nor sex differences were found. Data are presented as mean ± SD.

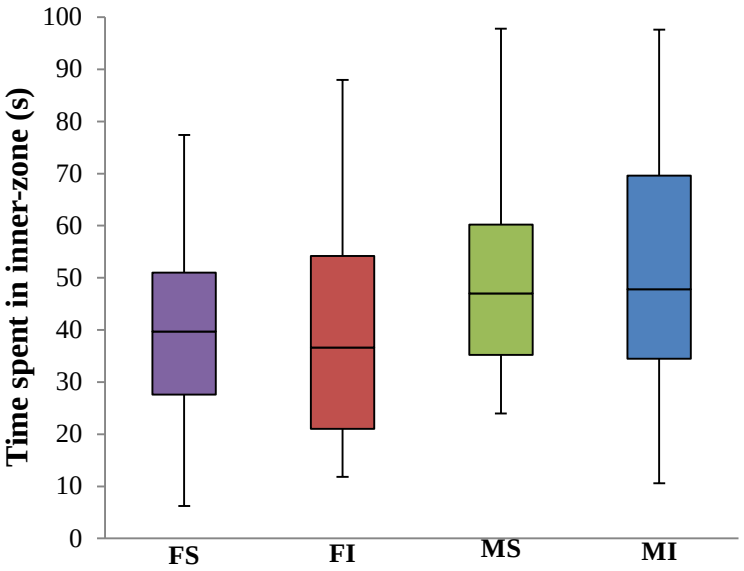

Figure 12- NOR P.1 5 MIN, Time spent in the inner-zone (s)

Time spent in the inner-zone (s) during open field exploration (phase 1) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data are presented as median ± IQR with min and max values.

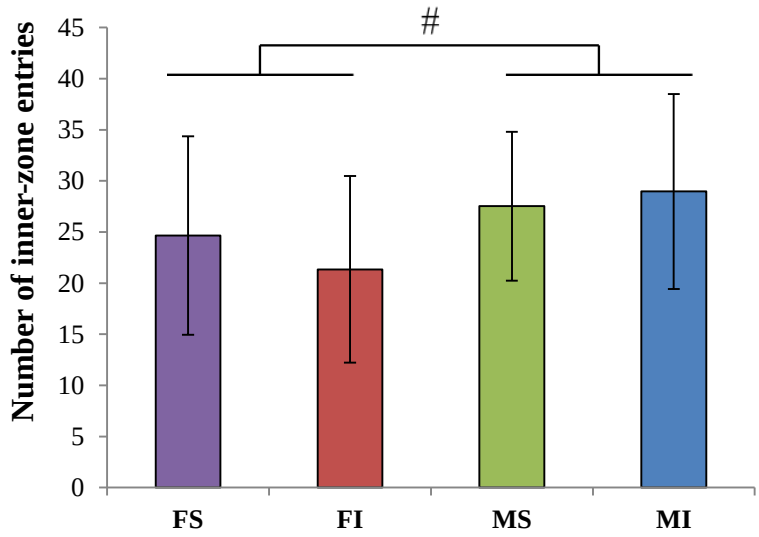

Figure 13- NOR P.1 5 MIN, Number of inner-zone entries

Number of entries into the inner-zone during open field exploration (phase 1) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and

male isolated (MI n=24). #A sex difference was found, p=0.0051, where males were found to enter the inner-zone more frequently than females, p=0.0055. Data are presented as mean ± SD.

3.2.1.3 P.1- TEN MINUTES

Data from the full ten minutes of the open field exploration trial (phase 1) were analysed, (n=97, 24 FS, 23 FI, 25 MS, 25 MI). All variables from phase 1 were tracked using centre-point detection (Figure 2.1). The following parameters were analysed; distance travelled in the arena (cm), time spent within the inner-zone (s) and number of entries into the inner-zone. Full statistical tables can be found in appendix A.2.1.3.

Neither housing nor sex differences were found for distance travelled (cm) (Figure 14). For the time spent in the inner-zone (s) (Figure 15), a sex difference was found ($F_{(1, 93)}$ = 7.805, p=0.0063), where males spent more time in the inner-zone than females, p=0.0062. For the number of entries made into the inner-zone (Figure 16), a sex difference was found ($F_{(1, 93)}$ = 11.17, p=0.0011), where males made more entries into the inner-zone than females, p=0.0012.

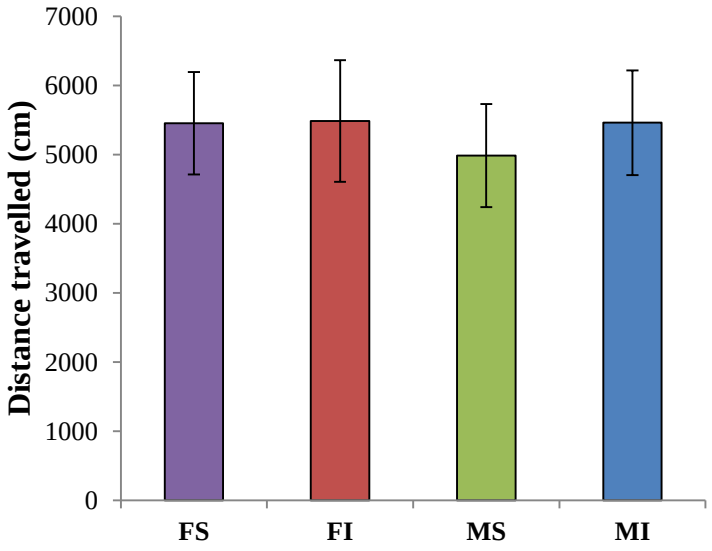

Figure 14- NOR P.1 10 MIN, Distance travelled (cm)

Distance travelled (cm) during open field exploration (phase 1) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male

isolated (MI n=25). Neither housing nor sex differences were found. Data are presented as mean ± SD.

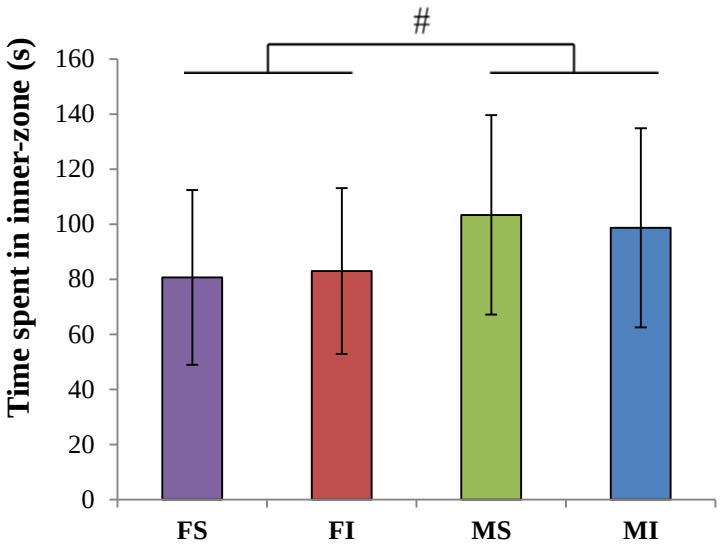

Figure 15- NOR P.1 10 MIN, Time spent in the inner-zone (s)

Time spent in the inner-zone (s) during open field exploration (phase 1) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=25). #A sex difference was found, p=0.0063, where males spent more time in the inner-zone than females, p=0.0062. Data are presented as mean ± SD.

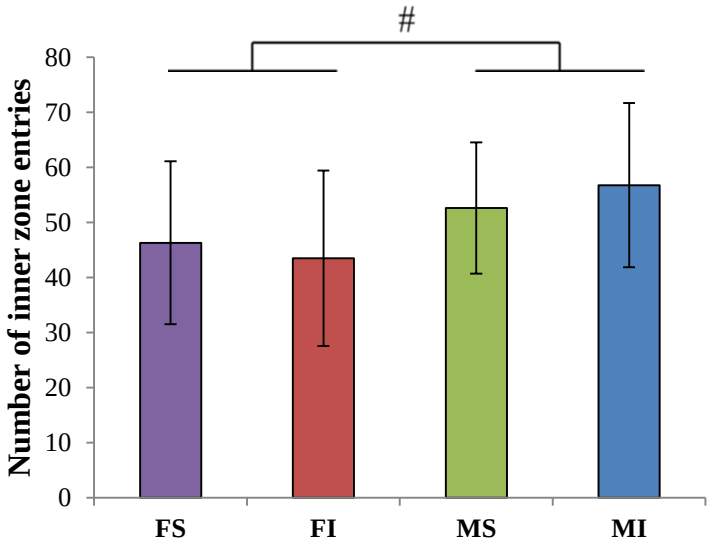

Figure 16- NOR P.1 10 MIN, Number of inner-zone entries

Number of entries into the inner-zone during open field exploration (phase 1) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and

male isolated (MI n=25). #A sex difference was found, p=0.0011, where males were found to enter the inner-zone more frequently than females, p=0.0012. Data are presented as mean ± SD.

3.2.2 PHASE 2- OBJECT FAMILIARISATION ANALYSIS

3.2.2.1 P.2- FIRST MINUTE

Data from the first minute of the object familiarisation trial (phase 2) were analysed, (n=95, 24 FS, 22 FI, 25 MS, 24 MI). In this trial two identical objects (O1, O2) were placed in respective quadrants (Q1, Q2). The following parameters were analysed; distance travelled in the arena (cm), time spent (s) in quadrants containing identical objects (Quadrants 1+2) vs. quadrants without objects (Quadrants 3+4), time spent (s) in each of the quadrants containing identical objects (Quadrant 1 vs. Quadrant 2), number of entries into each of the quadrants containing identical objects (Quadrant 1 vs. Quadrant 2), time spent (s) with each identical object (Object 1 vs. Object 2), number of approaches to each identical object (Object 1 vs. Object 2), and latency (s) to the first approach of each identical object (Object 1 vs. Object 2). Object variables were tracked by nose-point detection; all other variables were tracked by centre-point detection, see methodology for details (Figure 2.2). Preliminary analysis of the data showed that there was a preference for one of the objects or quadrants in the following tests; Quadrant 1 vs. Quadrant 2 (time spent, s), Object 1 vs. Object 2 (time spent, s) and Object 1 vs. Object 2 (number of approaches). This was taken as a bias in the experimental set up, therefore these variables were normalised using a correction factor calculated for each animal to compensate for this bias so that the data were comparable (Equation 2). Full statistical tables can be found in appendix A.2.2.1.

For distance travelled (cm) (Figure 17), a sex difference was found ($F_{(1,\ 91)}$ =32.54, p<0.0001), where females travelled further than males, p<0.0001. When comparing the time spent (s) in object-containing quadrants (Q1+Q2) to the time spent in object-free quadrants (Q3+Q4) (Figure 18), a difference was found ($\chi^2_{(1,\ N=95)}$ =43.57, p<0 .0001), where more time was spent in the object-containing quadrants than in the object-free quadrants. This difference was apparent for all groups; FS, p=0.0027, FI, p=0.0006, MS, p=0.0082 and MI p= 0.0007. Neither housing nor sex differences were found when comparing the time spent (s) in each of the quadrants containing identical objects (Q1 vs. Q2) (Figure 19). Neither housing nor sex differences were found when comparing the number of entries into each of the quadrants containing identical objects (Q1 vs. Q2) (Figure 20).

Neither housing nor sex differences were found when comparing the time spent (s) with each identical object (O1 vs. O2) (Figure 21). Neither housing nor sex differences were found when comparing the number of approaches to each identical object (O1 vs. O2) (Figure 22). Neither housing nor sex differences were found when comparing the latency (s) to the first approach of each identical object (O1 vs. O2) (Figure 23).

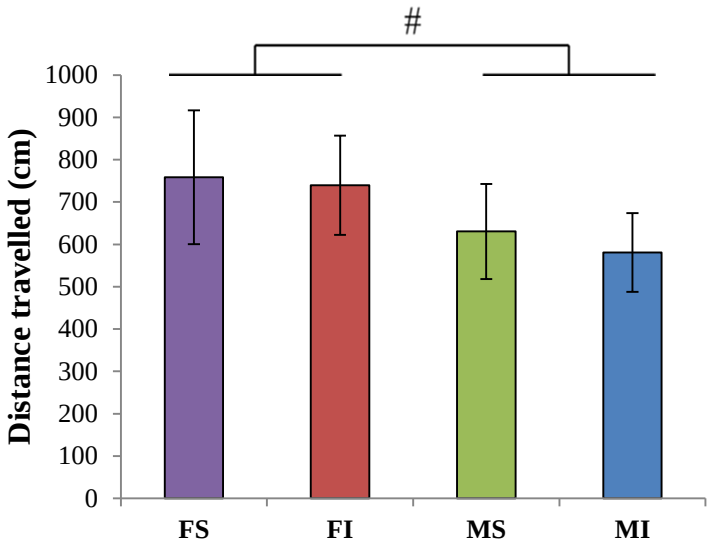

Figure 17- NOR P.2 1 MIN, Distance travelled (cm)

Distance travelled (cm) during object familiarisation (phase 2) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). #A sex difference was found, $p<0.0001$, where females travelled further than males, $p<0.0001$. Data are presented as mean ± SD.

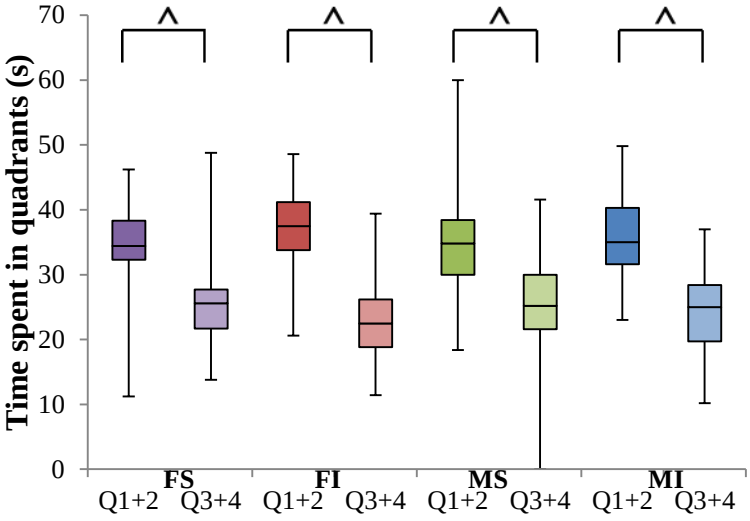

Figure 18- NOR P.2 1 MIN, Quadrants 1+2 vs. Quadrants 3+4 (s)

Time spent (s) during object familiarisation (phase 2) in object-containing quadrants (Q1+2) vs. time spent in object-free quadrants (Q3+4) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). ^ A difference was found, $p<0.0001$, where more time was spent in the object-containing quadrants than in the object-free quadrants. This result was apparent for all groups; FS, p=0.0027, FI, p=0.0006, MS, p=0.0082, MI, p= 0.0007. Data are presented as median ± IQR with min and max values. Q= quadrant, 1+2= object-containing quadrants, 3+4= object-free quadrants.

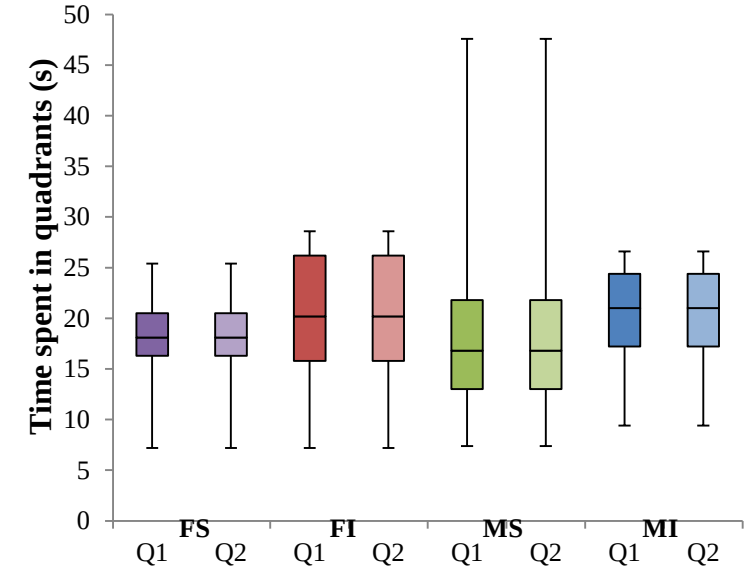

Figure 19- NOR P.2 1 MIN, Quadrant 1 vs. Quadrant 2 (s)

Time spent (s) during object familiarisation (phase 2) in quadrants containing identical objects (Q1 vs. Q2) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=22),

male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data were corrected, as phase 2 was familiarisation to objects in preparation for placement of a novel object in phase 3, time spent in Q1 was made equal to the time spent in Q2 for each animal, thus normalising preference for the identical object-containing quadrants at baseline. Data are presented as median ± IQR with min and max values. Q= quadrant, 1,2 = quadrants containing identical objects.

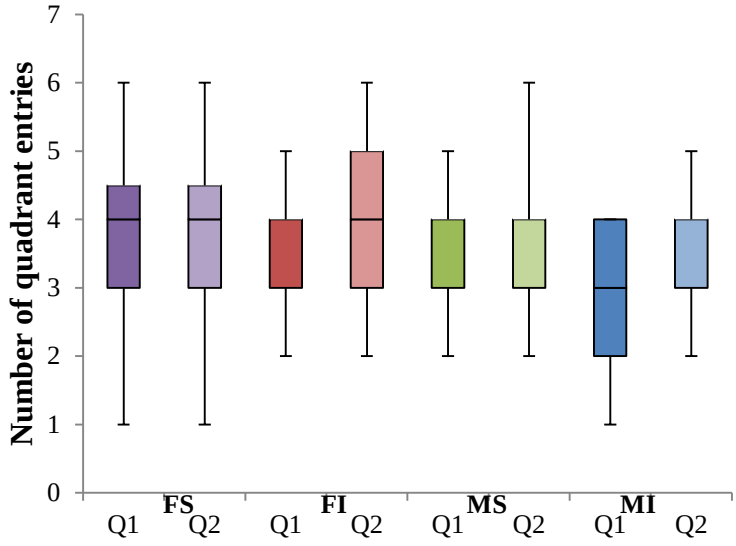

Figure 20- NOR P.2 1 MIN, Quadrant 1 vs. Quadrant 2 (number of entries)

Number of entries during object familiarisation (phase 2) into quadrants containing identical objects (Q1 vs. Q2) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data are presented as median ± IQR with min and max values. Q= quadrant, 1,2 = quadrants containing identical objects.

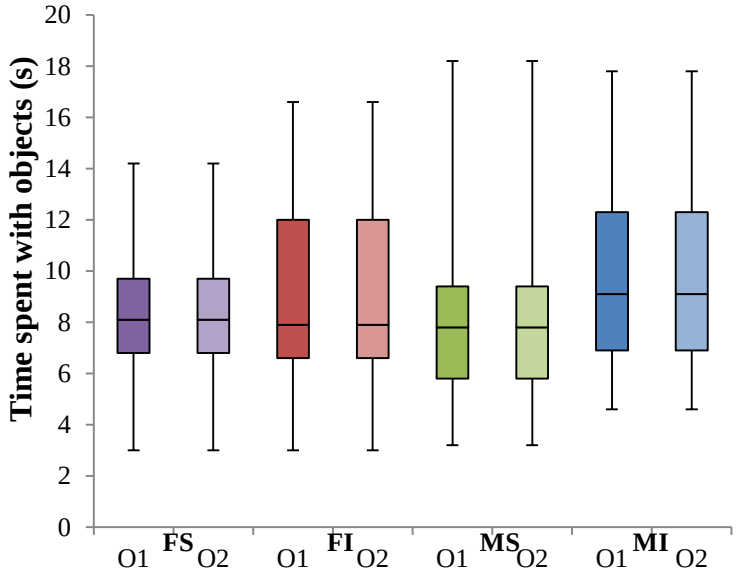

Figure 21- NOR P.2 1 MIN, Object 1 vs. Object 2 (s)

Time spent (s) during object familiarisation (phase 2) with each identical object (O1 vs. O2) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found Data were corrected, as phase 2 was familiarisation to objects in preparation for placement of a novel object in phase 3, time spent in O1 was made equal to the time spent in O2 for each animal, thus normalising preference for the identical objects at baseline. Data are presented as median ± IQR with min and max values. O = object, 1,2 = identical objects.

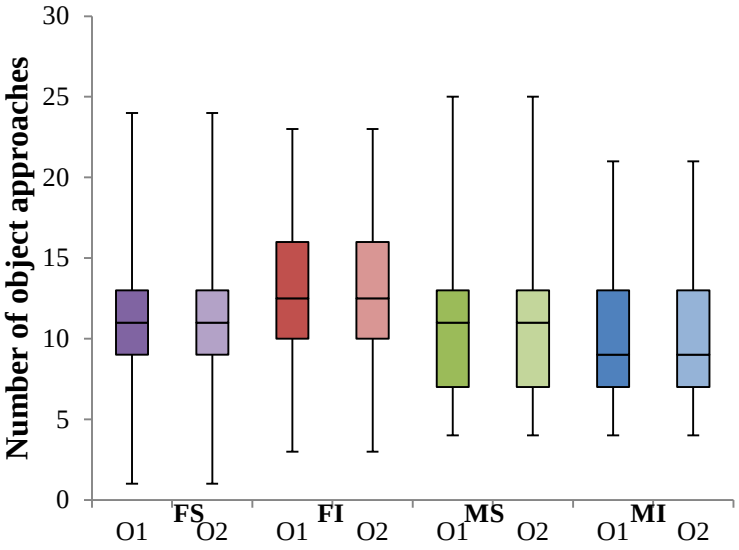

Figure 22- NOR P.2 1 MIN, Object 1 vs. Object 2 (number of approaches)

Number of approaches during object familiarisation (phase 2) to each identical object (O1 vs. O2) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data were corrected, as phase 2 was familiarisation to objects in preparation for placement of a novel object in phase 3, time spent in O1 was made equal to the time spent in O2 for each animal, thus normalising preference for the identical objects at baseline. Data are presented as median ± IQR with min and max values. O = object, 1,2 = identical objects.

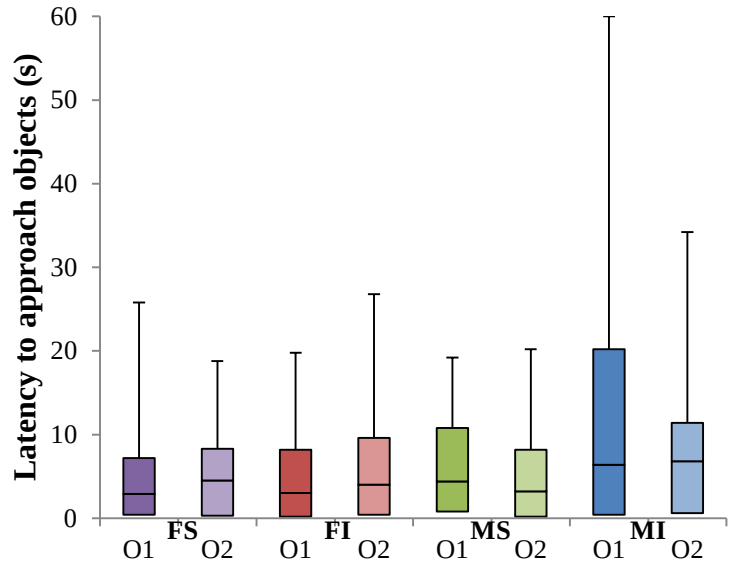

Figure 23- NOR P.2 1 MIN, Object 1 vs. Object 2 (latency to approach, s)

Latency to the first approach (s) during object familiarisation (phase 2) to each identical object (O1 vs. O2) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=22),

male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data are presented as median ± IQR with min and max values. O = object, 1,2 = identical objects.

3.2.2.2 P.2- FIVE MINUTES

Data from the first five minutes of the object familiarisation trial (phase 2) were combined and analysed, (n=95, 24 FS, 22 FI, 25 MS, 24 MI). In this trial two identical objects (O1, O2) were placed in respective quadrants (Q1, Q2). The following parameters were analysed; distance travelled in the arena (cm), time spent (s) in quadrants containing identical objects (Quadrants 1+2) vs. quadrants without objects (Quadrants 3+4), time spent (s) in each of the quadrants containing identical objects (Quadrant 1 vs. Quadrant 2), number of entries into each of the quadrants containing identical objects (Quadrant 1 vs. Quadrant 2), time spent (s) with each identical object (Object 1 vs. Object 2) and number of approaches to each identical object (Object 1 vs. Object 2). Object variables were tracked by nose-point detection; all other variables were tracked by centre-point detection, see methodology for details (Figure 2.2). Preliminary analysis of the data showed that there was a preference for one of the objects or quadrants in the following tests; Quadrant 1 vs. Quadrant 2 (time spent, s), Object 1 vs. Object 2 (time spent, s) and Object 1 vs. Object 2 (number of approaches), this preference was consistent with data from the analysis of the first minute. This was taken as a bias in the experimental set up, therefore these variables were normalised using a correction factor calculated for each animal to compensate for this bias so that the data were comparable (Equation 2). Full statistical tables can be found in appendix A.2.2.2.

For distance travelled (cm) (Figure 24), a sex difference was found ($F_{(1, 91)}$ = 38.73, $p<0.0001$), where females travelled further than males, $p<0.0001$. When comparing the time spent (s) in the object-containing quadrants (Q1+Q2) to the time spent in the object-free quadrants (Q3+Q4) (Figure 25), a difference was found; ($F_{(1, 91)}$ = 225.8, $p<0.0001$) where more time was spent in object-containing quadrants than in object-free quadrants, $p<0.0001$. Neither housing nor sex differences were found when comparing the time spent (s) in each of the quadrants containing identical objects (Q1 vs. Q2) (Figure 26). When comparing the number of entries into each of the quadrants containing identical objects (Q1 vs. Q2) (Figure 27), a sex difference was found ($F_{(1, 91)}$ = 18.90, $p<0.0001$), where females made more entries into both quadrants (Q1 + Q2) than males $p<0.0001$. Neither housing nor sex differences were found when comparing the time spent (s) with

each identical object (O1 vs. O2) (Figure 28). Neither housing nor sex differences were found when comparing the number of approaches to each of the identical objects (O1 vs. O2) (Figure 29).

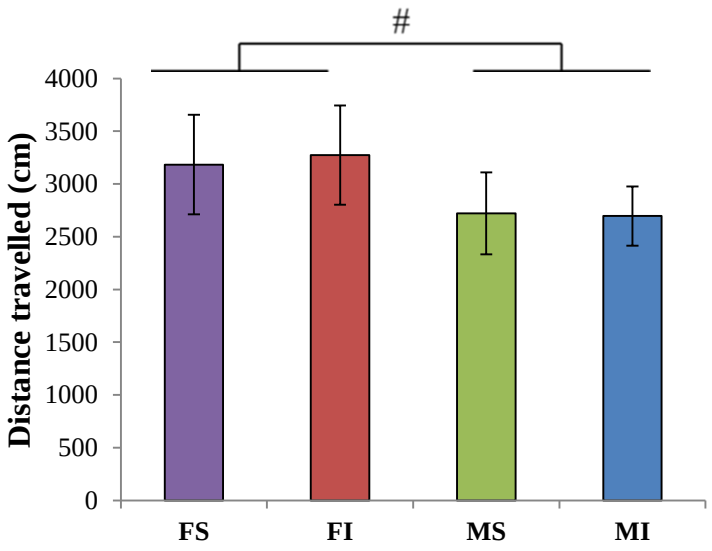

Figure 24- NOR P.2 5 MIN, Distance travelled (cm)

Distance travelled (cm) during object familiarisation (phase 2) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). #A sex difference was found, $p<0.0001$, where females travelled further than males, $p<0.0001$. Data are presented as mean ± SD.

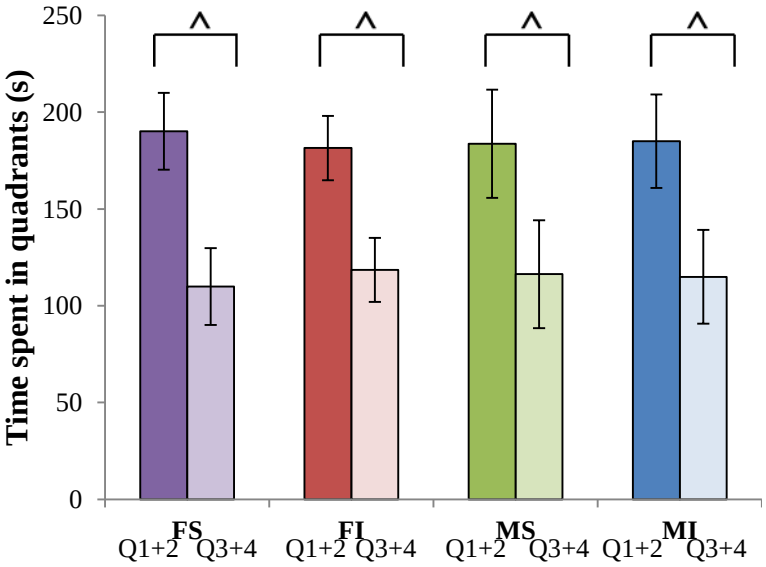

Figure 25- NOR P.2 5 MIN, Quadrants 1+2 vs. Quadrants 3+4 (s)

Time spent (s) during object familiarisation (phase 2) in object-containing quadrants (Q1+2) vs. time spent in object-free quadrants (Q3+4) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). ^A difference was found, $p<0.0001$, where more time was spent in the object-containing quadrants than in the object-free quadrants, $p<0.0001$. Data are presented as mean ± SD. Q= quadrant, 1+2= object containing quadrants, 3+4= object-free quadrants.

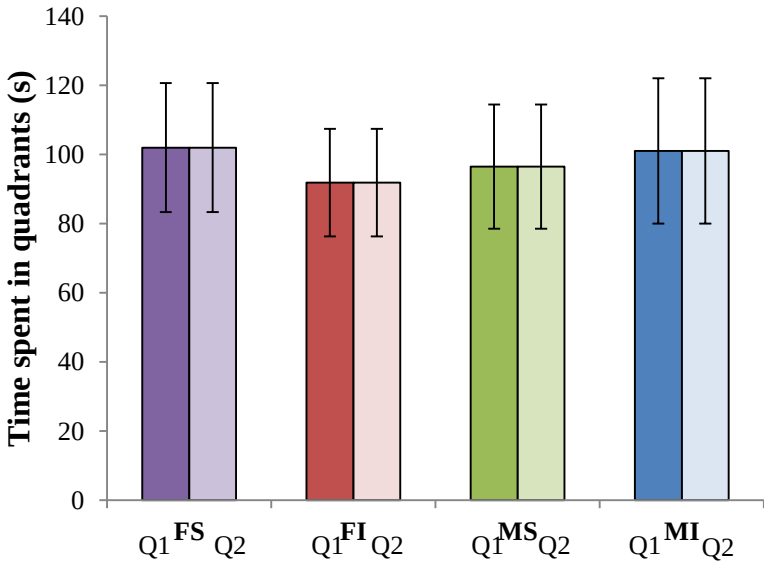

Figure 26- NOR P.2 5 MIN, Quadrant 1 vs. Quadrant 2 (s)

Time spent (s) during object familiarisation (phase 2) in quadrants containing identical objects (Q1 vs. Q2) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data were corrected, as phase 2 was familiarisation to objects in preparation for placement of a novel object in phase 3, time spent in Q1 was made equal to the time spent in Q2 for each animal, thus normalising preference for the identical object-containing quadrants at baseline. Data are presented as mean ± SD. Q= quadrant, 1, 2 = quadrants containing identical objects.

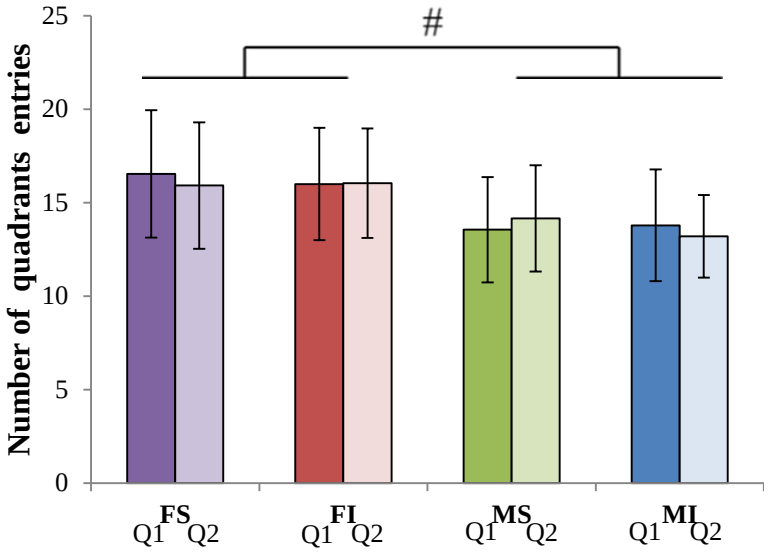

Figure 27- NOR P.2 5 MIN, Quadrant 1 vs. Quadrant 2 (number of entries)

Number of entries during object familiarisation (phase 2) into quadrants containing identical objects (Q1 vs. Q2) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). #A sex difference was found, $p<0.0001$, where females made more entries into both quadrants (Q1 + Q2) than males, $p<0.0001$. Data are presented as mean ± SD. Q= quadrant, 1, 2 = quadrants containing identical objects.

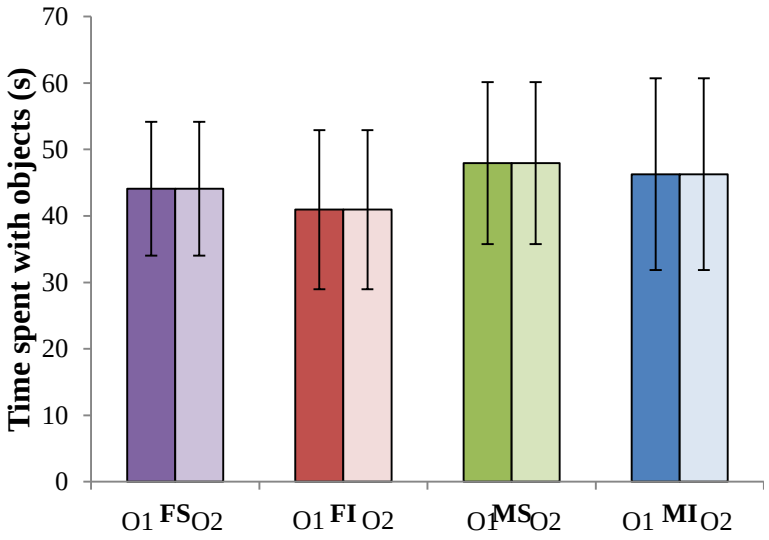

Figure 28- NOR P.2 5 MIN, Object 1 vs. Object 2 (s)

Time spent (s) during object familiarisation (phase 2) with each identical object (O1 vs. O2) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data were corrected, as phase 2 was familiarisation to objects in preparation for placement of a novel object in

phase 3, time spent in O1 was made equal to the time spent in O2 for each animal, thus normalising preference for the identical object-containing quadrants at baseline. Data are presented as mean ± SD. O = object, 1, 2 = identical objects.

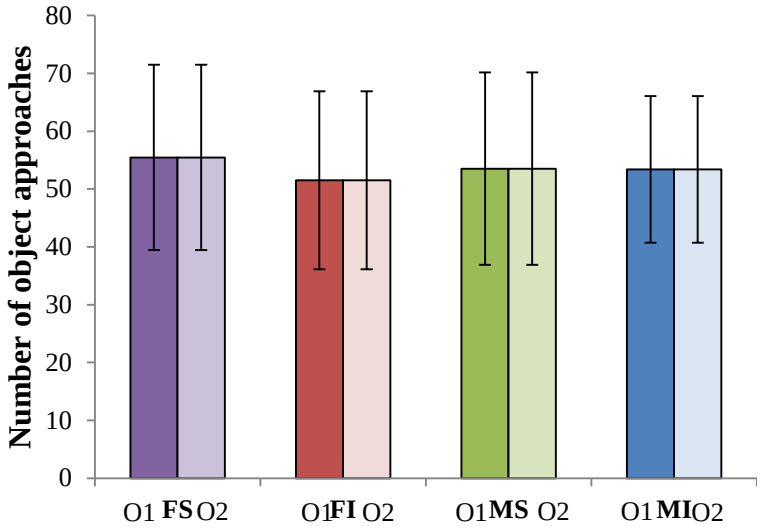

Figure 29- NOR P.2 5 MIN, Object 1 vs. Object 2 (number of approaches)

Number of approaches during object familiarisation (phase 2) to each identical object (O1 vs. O2) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data were corrected, as phase 2 was familiarisation to objects in preparation for placement of a novel object in phase 3, time spent in O1 was made equal to the time spent in O2 for each animal, thus normalising preference for the identical objects at baseline. Data are presented as mean ± SD. O = object, 1,2 = identical objects.

3.2.3 PHASE 3- NOVEL OBJECT RECOGNITION ANALYSIS

3.2.3.1 P.3- FIRST MINUTE

Data from the first minute of the novel object recognition trial (phase 3) were analysed, (n=96, 24 FS, 23 FI, 25 MS, 24 MI). In this trial object 1 (O1) in quadrant 1 (Q1) was a novel item whilst object 2 (O2) in quadrant 2 (Q2) remained the same as in the previous trial (phase 2). The correction factor, calculated for each animal in phase 2 where a behavioural apparatus bias was evident (Equation 2), was applied to the corresponding variables in phase 3. The following parameters were analysed; distance travelled in the arena (cm), time spent (s) in each of the

quadrants containing different objects (Quadrant 1 vs. Quadrant 2), number of entries into each of the quadrants containing different objects (Quadrant 1 vs. Quadrant 2), quadrant discrimination index, time spent (s) with each identical object (Object 1 vs. Object 2), number of approaches to each identical object (Object 1 vs. Object 2), latency (s) to the first approach of each identical object (Object 1 vs. Object 2) and object discrimination index. Object variables were tracked by nose-point detection; all other variables were tracked by centre-point detection, (Figure 2.3) see methodology for details. Full statistical tables can be found in appendix A.2.3.1.

For distance travelled (cm) (Figure 30), a main effect of housing was found ($F_{(1,\ 92)}$ =9.210, p=0.0031), where socialised animals travelled further than isolated animals, p=0.0029. A sex difference was also found for the distance travelled (cm) ($F_{(1,\ 92)}$ =83.10, p<0.0001), where females travelled further than males, p<0.0001. When comparing the time spent (s) in novel object quadrant (Q1) to time spent in familiar object quadrant (Q2) (Figure 31), a difference was found ($\chi^2_{(1,\ N\ =\ 94)}$ =6.127, p=0.0133), where both FI and MI spent more time in Q1 than in Q2, p=0.008 and p=0.0386 respectively. Neither housing nor sex differences were found when comparing the number of entries into novel object quadrant (Q1) to the number of entries into familiar quadrant (Q2) (Figure 32). Neither housing nor sex differences were found for the quadrant discrimination index (Figure 33). When comparing the time spent (s) with the novel object (O1) to time spent with the familiar object (O2) (Figure 34), a difference was found ($\chi^2_{(1,\ N\ =\ 94)}$ =19.88, p < 0.0001), where all groups spent more time with novel object than with the familiar object; FS, p=0.0240, FI, p=0.0004, MS, p=0.0060 and MI, p=0.0011. When comparing number of approaches to the novel object (O1) to number of approaches to the familiar object (O2) (Figure 35), a difference was found ($\chi^2_{(1,\ N\ =\ 94)}$ = 15.69, p < 0.0001), where all groups made more approaches to the novel object than the familiar object; FS, p=0.0162, FI, p=0.0015, MS, p=0.0109 and MI, p=0.0115. Neither housing nor sex differences were found when comparing the latency (s) to the first approach of the novel object (O1) area with the latency to the first approach of the familiar object (O2) area (Figure 36). Neither housing nor sex differences were found for the object discrimination index (Figure 37).

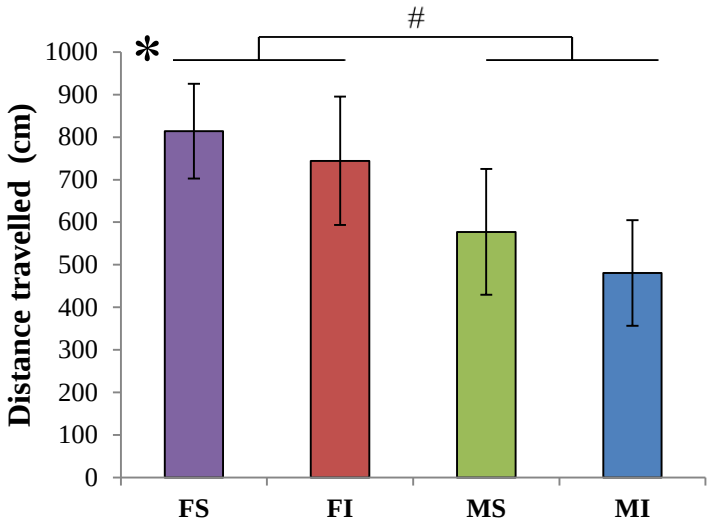

Figure 30- NOR P.3 1 MIN, Distance travelled (cm)

Distance travelled (cm) during novel object recognition (phase 3) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=24). * A main effect of housing was found, p=0.0031, where socialised animals travelled further than isolated animals, p=0.0029. #A sex difference was found, p<0.0001, where females travelled further than males, p<0.0001. Data are presented as mean ± SD

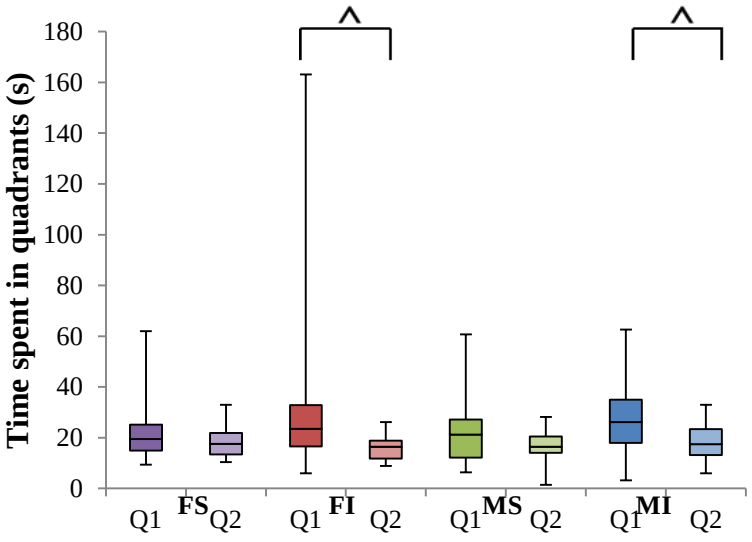

Figure 31- NOR P.3 1 MIN, Quadrant 1 vs. Quadrant 2 (s)

Time spent (s) during novel object recognition (phase 3) in novel object quadrant (Q1) vs. time spent in familiar object quadrant (Q2) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=23). ^ A difference

was found, p = 0.0133, where more time was spent in the novel object quadrant than in the familiar object quadrant. This was apparent for the two isolate groups; FI, p=0.008 and MI, p=0.0386. Data were corrected such that Q1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as median ± IQR with min and max values. Q= quadrant, 1= quadrant containing novel object, 2= quadrant containing familiar object.

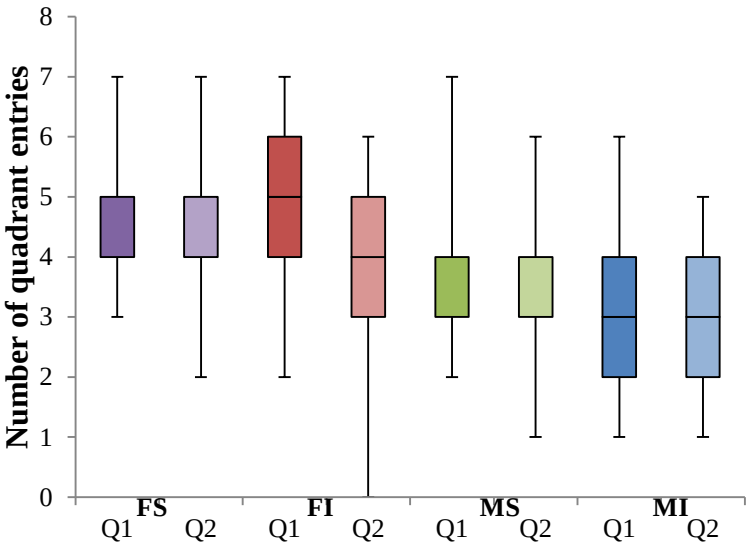

Figure 32- NOR P.3 1 MIN, Quadrant 1 vs. Quadrant 2 (number of entries)

Number of entries during novel object recognition (phase 3) into novel object quadrant (Q1) vs. number of entries into familiar object quadrant (Q2) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data are presented as median ± IQR with min and max values. Q= quadrant, 1= quadrant containing novel object, 2= quadrant containing familiar object.

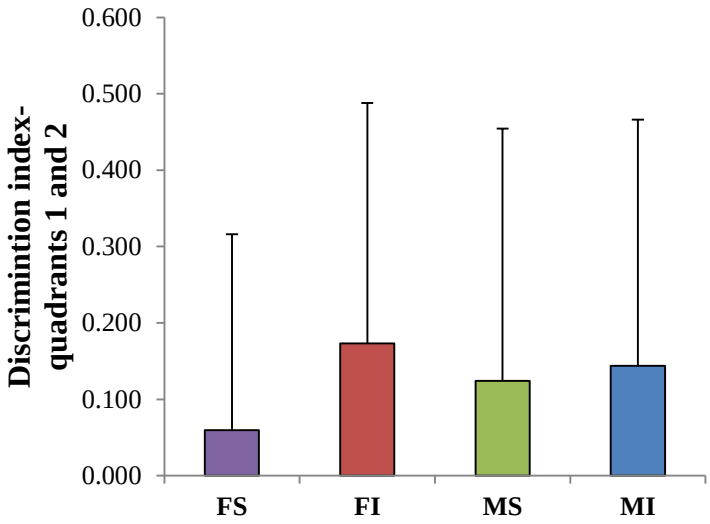

Figure 33- NOR P.3 1 MIN, Quadrant discrimination index

Discrimination index calculated from the time spent in novel object quadrant (Q1) and familiar object quadrant (Q2) during novel object recognition (phase 3) (see methodology for calculation), for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=23). Neither housing nor sex differences were found. Data were corrected such that Q1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as mean + SD.

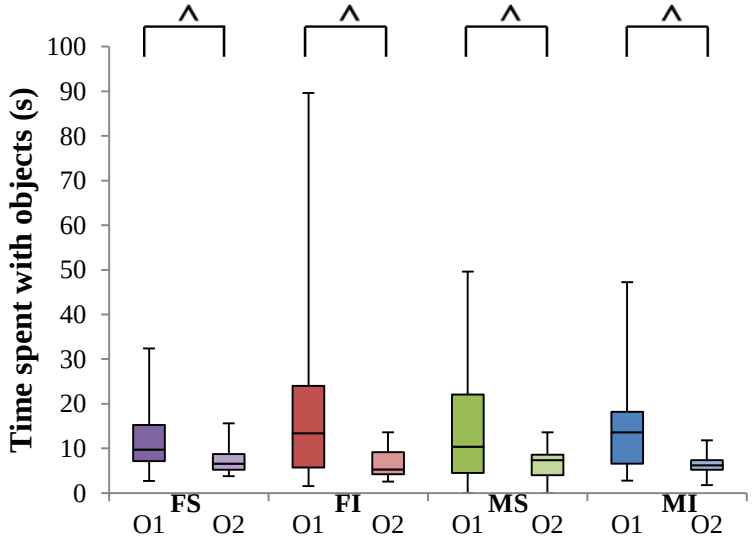

Figure 34- NOR P.3 1 MIN, Object 1 vs. Object 2 (s)

Time spent (s) during novel object recognition (phase 3) with novel object (O1) vs. time spent with familiar object (O2) for each sex_housing group; female socialised (FS n=24) and female isolated

(FI n=22), male socialised (MS n=25) and male isolated (MI n=23). ^A difference was found, p<0.0001; where all groups spent more time with the novel object than with the familiar object; FS, p=0.0240, FI, p=0.0004, MS, p=0.0060 and MI, p=0.0011. Data were corrected such that O1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as median ± IQR with min and max values. O = object, 1= novel object, 2= familiar object.

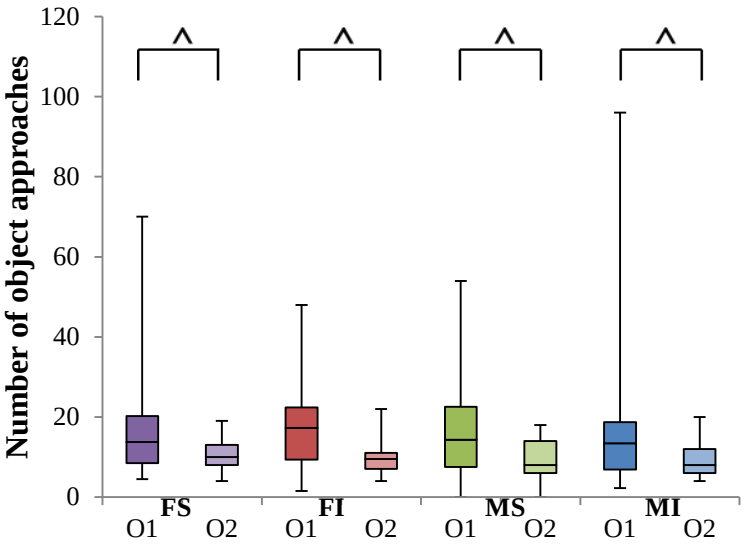

Figure 35- NOR P.3 1 MIN, Object 1 vs. Object 2 (number of approaches)

Number of approaches during novel object recognition (phase 3) to novel object (O1) vs. number of approaches to familiar object (O2) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=23). ^A difference was found, p=0.0001, where all groups made more approaches to the novel object than the familiar object; FS, p=0.0162, FI, p=0.0015, MS, p=0.0109 and MI, p=0.0115. Data were corrected such that O1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as median ± IQR with min and max values. O = object, 1= novel object, 2= familiar object.

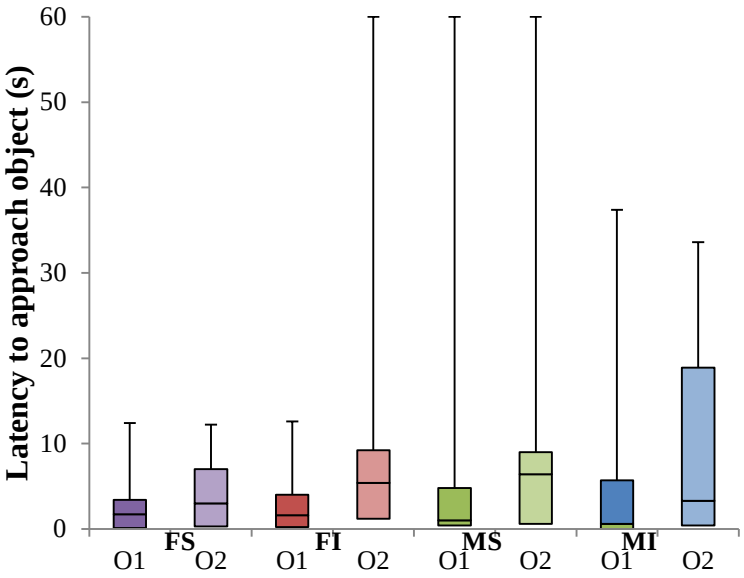

Figure 36- NOR P.3 1 MIN, Object 1 vs. Object 2 (latency to approach, s)

Latency to the first approach (s) during novel object recognition (phase 3) to novel object (O1) vs. latency to the first approach to the familiar object (O2) for each sex_housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=24). Neither housing nor sex differences were found. Data are presented as median ± IQR with min and max values. O = object, 1= novel object, 2= familiar object.

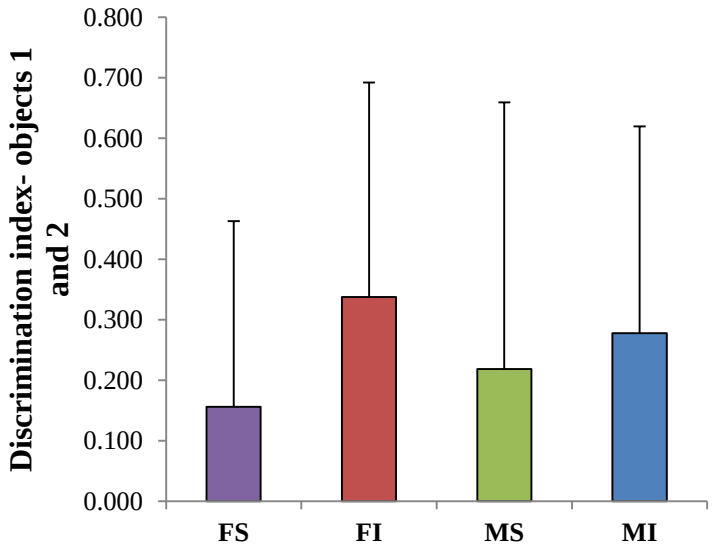

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Figure 37- NOR P.3 1 MIN, Object discrimination index

Discrimination index calculated from the time spent with novel object (O1) and familiar object (O2) during novel object recognition (phase 3) (see methodology for calculation) for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=22), male socialised (MS

n=25) and male isolated (MI n=23). Neither housing nor sex differences were found. Data were corrected such that Q1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as mean + SD.

3.2.3.2 P.3- FIVE MINUTES

Data from the first five minutes of the novel object recognition trial (phase 3) were analysed, (n=94, 23 FS, 23 FI, 25 MS, 23 MI). In this trial object 1 (O1) in quadrant 1 (Q1) was a novel item whilst object 2 (O2) in quadrant 2 (Q2) remained the same as in the previous trial (phase 2). The correction factor calculated for each animal in phase 2 where a behavioural apparatus bias was evident (Equation 2) was applied to the corresponding variables in phase 3. The following parameters were analysed; distance travelled in the arena (cm), time spent (s) in each of the quadrants containing different objects (Quadrant 1 vs. Quadrant 2), number of entries into each of the quadrants containing different objects (Quadrant 1 vs. Quadrant 2), quadrant discrimination index, time spent (s) with each identical object (Object 1 vs. Object 2), number of approaches to each identical object (Object 1 vs. Object 2) and object discrimination index. Object variables were tracked by nose-point detection; all other variables were tracked by centre-point detection, (Figure 2.3) see methodology for details. Full statistical tables can be found in appendix A.2.3.2.

For the distance travelled (cm) (Figure 38), a sex difference was found ($F_{(1, 90)} = 41.64$, $p<0.0001$), where female groups travelled further than male groups, $p<0.0001$. Neither housing nor sex differences were found when comparing the time spent (s) in the novel object quadrant (Q1) to the time spent in the familiar object quadrant (Q2) (Figure 39). When comparing the number of entries into the novel object quadrant (Q1) to the number of entries into the familiar object quadrant (Q2) (Figure 40), a sex difference was found ($F_{(1, 90)} = 20.39$, $p<0.0001$), where females made more entries into both quadrants (Q1+Q2) than males, $p<0.0001$. A housing-sex-quadrant interaction was also found for the number of entries into the quadrants ($F_{(1, 90)} = 5.30$, $p=0.0235$), where the FI made more entries into Q1 than MI Q1, $p=0.0010$ and MI Q2, $p=0.0071$. FI also made more entries into Q2 than MI Q1, $p=0.0095$. Neither housing nor sex differences were found for the quadrant discrimination index (Figure 41). When comparing the time spent (s) with the novel object (O1) to the time spent with the familiar object (O2) (Figure 42), a differences was found ($\chi^2_{(1, N= 92)} =17.39$, $p<0.0001$), where FI and MI both spent more time with O1 than O2, $p=0.0045$ and $p=0.0055$ respectively. When comparing the number of approaches to the novel object (O1) to the number of

approaches to the familiar object (O2) (Figure 43), a difference was found ($\chi^2_{(1,\ N=\ 92)}$ =9.782, p=0.0017), where FS, FI and MS all made more approaches to O1 than O2 where p=0.0264, p=0.0155 and p=0.0450 respectively. Neither housing nor sex differences were found for the object discrimination index (Figure 44). Novelty preference results are summarised in Table 8.

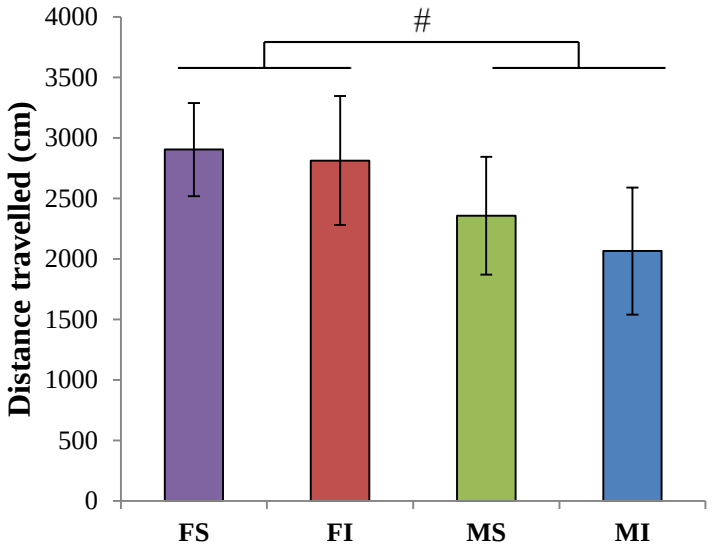

Figure 38- NOR P.3 5 MIN, Distance travelled (cm)

Distance travelled (cm) during novel object recognition (phase 3) for each sex_housing group; female socialised (FS n=23) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=23). #A sex difference was found, p<0.0001, where females travelled further than males, p<0.0001. Data are presented as mean ± SD.

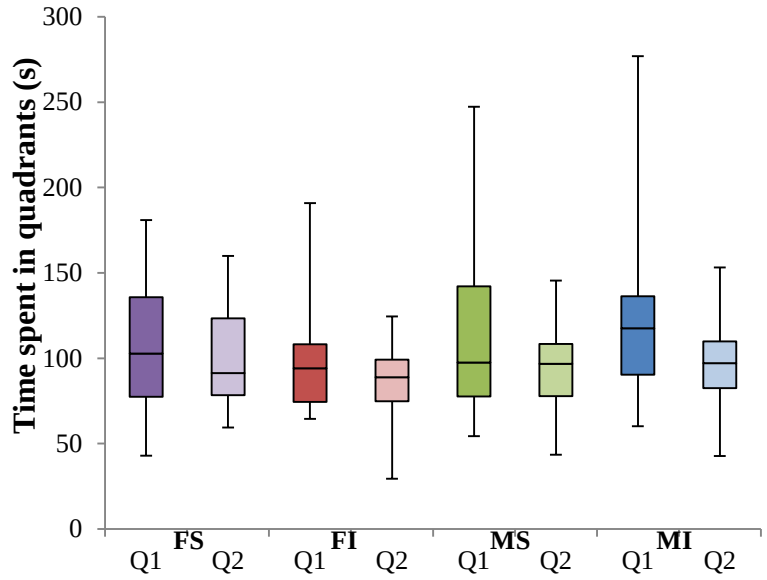

Figure 39- NOR P.3 5 MIN, Quadrant 1 vs. Quadrant 2 (s)

Time spent (s) during novel object recognition (phase 3) in novel object quadrant (Q1) vs. time spent in familiar object quadrant (Q2) for each sex_housing group; female socialised (FS n=23) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=22). Neither housing nor sex differences were found. Data were corrected such that Q1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as median ± IQR with min and max values. Q= quadrant, 1= quadrant containing novel object, 2= quadrant containing familiar object.

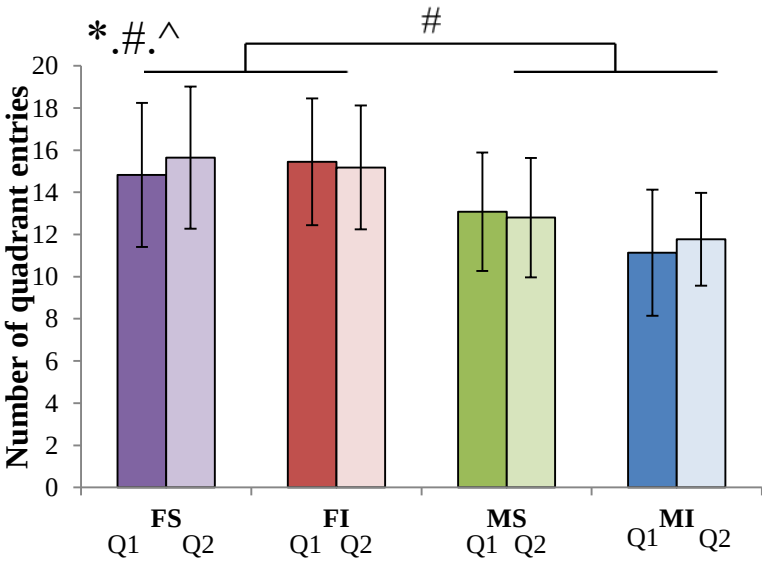

Figure 40- NOR P.3 5 MIN, Quadrant 1 vs. Quadrant 2 (number of entries)

Number of entries during novel object recognition (phase 3) into novel object quadrant (Q1) vs. number of entries into familiar object quadrant (Q2) for each sex and housing group; female socialised (FS n=23) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=23). [#]A sex difference was found, p<0.0001; where females made more entries into both quadrants (Q1 + Q2) than males, p<0.0001. [*.#.^] A housing-sex-quadrant difference was also found, p=0.0235 (Table 70 for post hoc values). Data are presented as mean ± SD. Q= quadrant, 1= quadrant containing novel object, 2= quadrant containing familiar object.

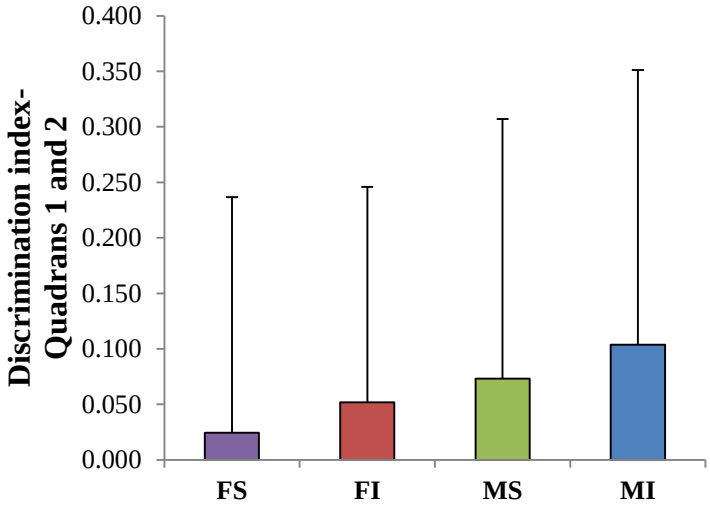

Figure 41- NOR P.3 5 MIN, Quadrant discrimination index

Discrimination index calculated from the time spent in novel object quadrant (Q1) and familiar object quadrant (Q2) during novel object recognition (phase 3) (see methodology for calculation) for each sex and housing group; female socialised (FS n=23) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=22). Neither housing nor sex differences were found. Data were corrected such that Q1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as mean ± SD.

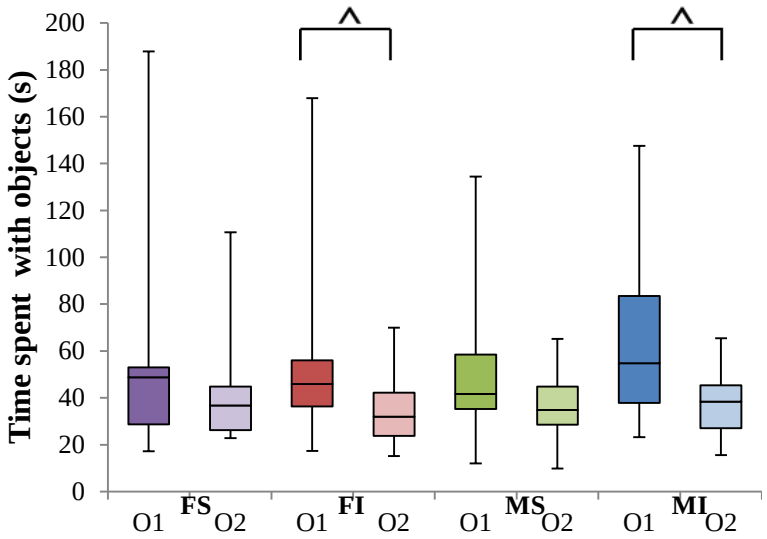

Figure 42- NOR P.3 5 MIN, Object 1 vs. Object 2 (s)

Time spent (s) during novel object recognition (phase 3) with novel object (O1) vs. time spent with familiar object (O2) for each sex_housing group; female socialised (FS n=23) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=22). ^ A difference was found, $p<0.0001$, where FI and MI spent more time with novel object than familiar object, $p=0.0045$ and $p=0.0055$ respectively. Data were corrected such that O1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as median ± IQR with min and max values. O = object, 1= novel object, 2= familiar object.

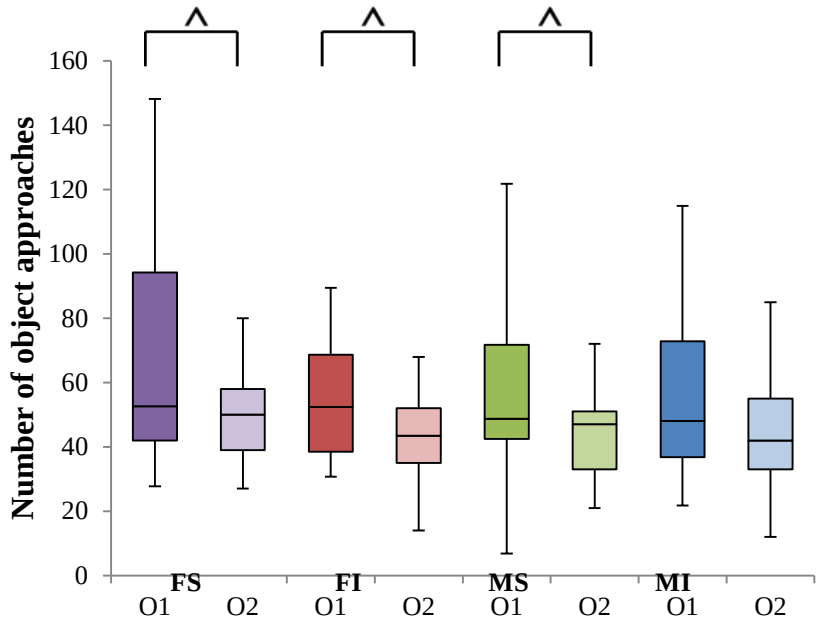

Figure 43- NOR P.3 5 MIN, Object 1 vs. Object 2 (number of approaches)

Number of approaches during novel object recognition (phase 3) to the novel object (O1) vs. number of approaches to the familiar object (O2) for each sex_housing group; socialised (FS n=23) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=22). ^ A difference was found, p=0.0017, where FS, FI and MS made more approaches to the novel object than the familiar object, p=0.0264, p=0.0155 and p=0.0450 respectively. Data were corrected such that O1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as median ± IQR with min and max values. O = Object, 1= novel object, 2= familiar object

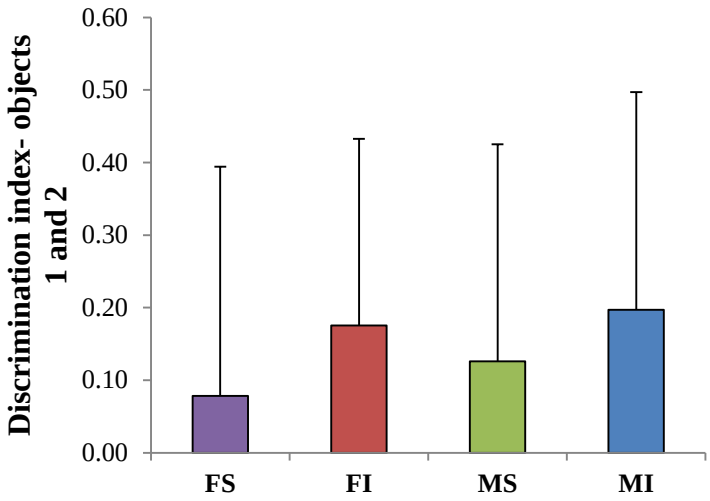

Figure 44- NOR P.3 5 MIN, Object discrimination index

Discrimination index calculated from the time spent with novel object (O1) and familiar object (O2) during novel object recognition (phase 3) (see methodology for calculation) for each sex and housing group; female socialised (FS n=23) and female isolated (FI n=22), male socialised (MS n=25) and male isolated (MI n=22).). Neither housing nor sex differences were found. Data were corrected such that O1 data were multiplied by the correction factor calculated in phase 2 for each animal (Equation 2), thus adjusting for preference at baseline. Data are presented as mean + SD.

Table 8- NOR P.3, Novelty preference summary

Groups listed showed preference for the novel quadrant or novel object as evidenced by time spent or how many entries or approaches were made.

	1 minute		**5 minute**	
	Time spent (s)	**Entries/approaches**	**Time spent (s)**	**Entries/approaches**
Novel Quadrant	FI MI			
Novel Object	FS, FI, MS, MI	FS, FI, MS, MI	FI, MI	FS, FI, MS

3.2.4 ALL PHASES- DISTANCE TRAVELLED

Distance travelled during the first minute and the first five minutes was measured in each phase. Comparisons were made between the phases to investigate whether the reaction to being in the

arena changed after repeated exposures to it. Findings from statistical tests applied to distance travelled data are summarised in Table 9 and Table 10.

3.2.4.1 P.1, P.2, P.3- FIRST MINUTE

Data for the distance travelled (cm) in the arena during the first minute were analysed across all three phases of the NOR test (n=96, 24 FS, 23 FI, 25 MS, 24 MI). Distance travelled was tracked by centre-point detection (Figure 2), see methodology for details. Full statistical tables can be found in appendix A.2.4.1.

For distance travelled (cm) in all three phases of the NOR test (Figure 45), a main effect of housing was found ($F_{(1,\ 90)}$ =9.497, p=0.0027), where socialised animals travelled further than isolated animals, p=0.0026. A sex difference was also found for the distance travelled (cm) in all three phases ($F_{(1,\ 90)}$ =73.74, p<0.0001), where females travelled further than males, p<0.0001. A difference between the phases was also found for the distance travelled (cm) ($F_{(2,\ 180)}$ =110.3, p<0.0001), where the distance travelled in phase 2 was greater than the distance travelled in phase 1, p<0.0001 and where the distance travelled in phase 3 was greater than the distance travelled in phase 1, p<0.0001. A sex-phase interaction was found for the distance travelled (cm) ($F_{(2,\ 180)}$ =19.74, p<0.0001), such that the distance travelled by females in phase 1 was greater than the distance travelled by males in phase 1, p=0.0457, the distance travelled by females in phase 2 was greater than the distance travelled by males in phase 2, p<0.0001, the distance travelled by females in phase 3 was greater than the distance travelled by males in phase 3, p<0.0001, the distance travelled by females in phase 2 was greater than the distance travelled by females in phase 1, p<0.0001, the distance travelled by females in phase 3 was greater than the distance travelled by females in phase 2, p<0.0001, the distance travelled by males in phase 2 was greater than the distance travelled by males in phase 1, p<0.0001, the distance travelled by males in phase 3 was greater than the distance travelled by males in phase 1, p=0.0003 and the distance travelled by males in phase 2 was greater than the distance travelled by males in phase 3, p<0.0001.Other sex-phase interactions were found but were not of statistical relevance (see appendix A3.2.1.4).

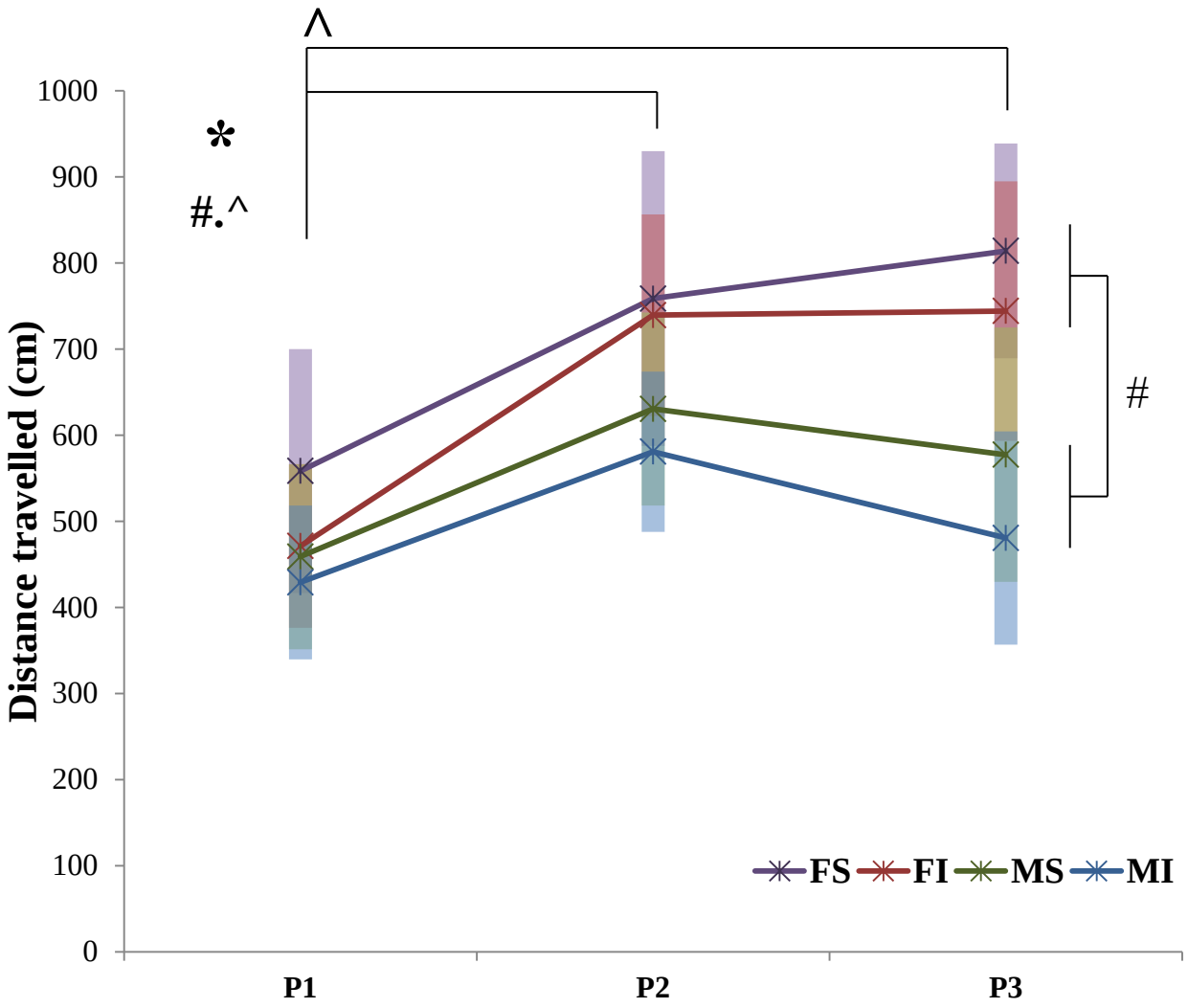

Figure 45- NOR All phases 1 MIN, Distance travelled (cm)

Distance travelled (cm) during first minute of all three phases of the NOR test for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=24). * A main effect of housing was found, p=0.0027, where socialised animals travelled further than isolated animals, p=0.0026. #A sex difference was found, p<0.0001, where females travelled further than males, p<0.0001. ^A difference between the phases was found, p<0.0001, where the distance travelled in phase 2 was greater than the distance travelled in phase 1, p<0.0001 and where the distance travelled in phase 3 was greater than the distance travelled in phase 1, p<0.0001. #.^ A sex-phase interaction was found, see text for p values. Data are presented as mean ± SD

3.2.4.2 P.1, P.2, P.3- FIVE MINUTES

Data for the distance travelled (cm) in the arena during the first five minutes were analysed across all three phases of the NOR test (n=96, 24 FS, 23 FI, 25 MS, 24 MI). Distance travelled was tracked by centre-point detection (Figure 2), see methodology for details. Full statistical tables can be found in appendix A.2.4.2.

For distance travelled (cm) in all three phases of the NOR test (Figure 46), a sex difference was found ($F_{(1,\ 88)}$ =38.79, p<0.0001), where females travelled further than males, p<0.0001. A difference between the phases was also found for the distance travelled (cm) ($F_{(2,\ 176)}$ =39.77, p<0.0001), where the distance travelled in phase 1 was greater than the distance travelled in phase 3, p<0.0001 and where the distance travelled in phase 2 was greater than the distance travelled in phase 3, p<0.0001. A sex-phase interaction was found for the distance travelled (cm) ($F_{(2,\ 176)}$ =12.95, p<0.0001), such that the distance travelled by females in phase 2 was greater than the distance travelled by males in phase 2, p<0.0001, the distance travelled by females in phase 3 was greater than the distance travelled by males in phase 3, p<0.0001,the distance travelled by females in phase 2 was greater than the distance travelled by females in phase 1, p=0.0010, the distance travelled by females in phase 2 was greater than the distance travelled by females in phase 3, p<0.0001, the distance travelled by males in phase 1 was greater than the distance travelled by males in phase 3 , p<0.0001 and the distance travelled by males in phase 2 was greater than the distance travelled by males in phase 3, p<0.0001. Other sex-phase interactions were found but were not of statistical relevance (see appendix A2.4.2).

Table 9- Hyperactivity summary within phases

The table lists sex or housing groups which demonstrated hyperactivity in each of the different phases and temporal analyses of the NOR test.

	1 minute		**5 minute**		**10 minute**	
	Sex	**Housing**	**Sex**	**Housing**	**Sex**	**Housing**
Phase 1	Females	Socialised				
Phase 2	Females		Females		n/a	n/a
Phase 3	Females	Socialised	Females		n/a	n/a

Table 10- Hyperactivity summary across phases

The table lists sex groups, housing groups or phases where hyperactivity was evident when data from all 3 phases were tested simultaneously.

1 minute			**5 minute**		
Sex	**Housing**	**Phase**	**Sex**	**Housing**	**Phase**
Females	Socialised	P.1>P.2 P.1>P.3	Females		P.1>P.3 P.2>P.3

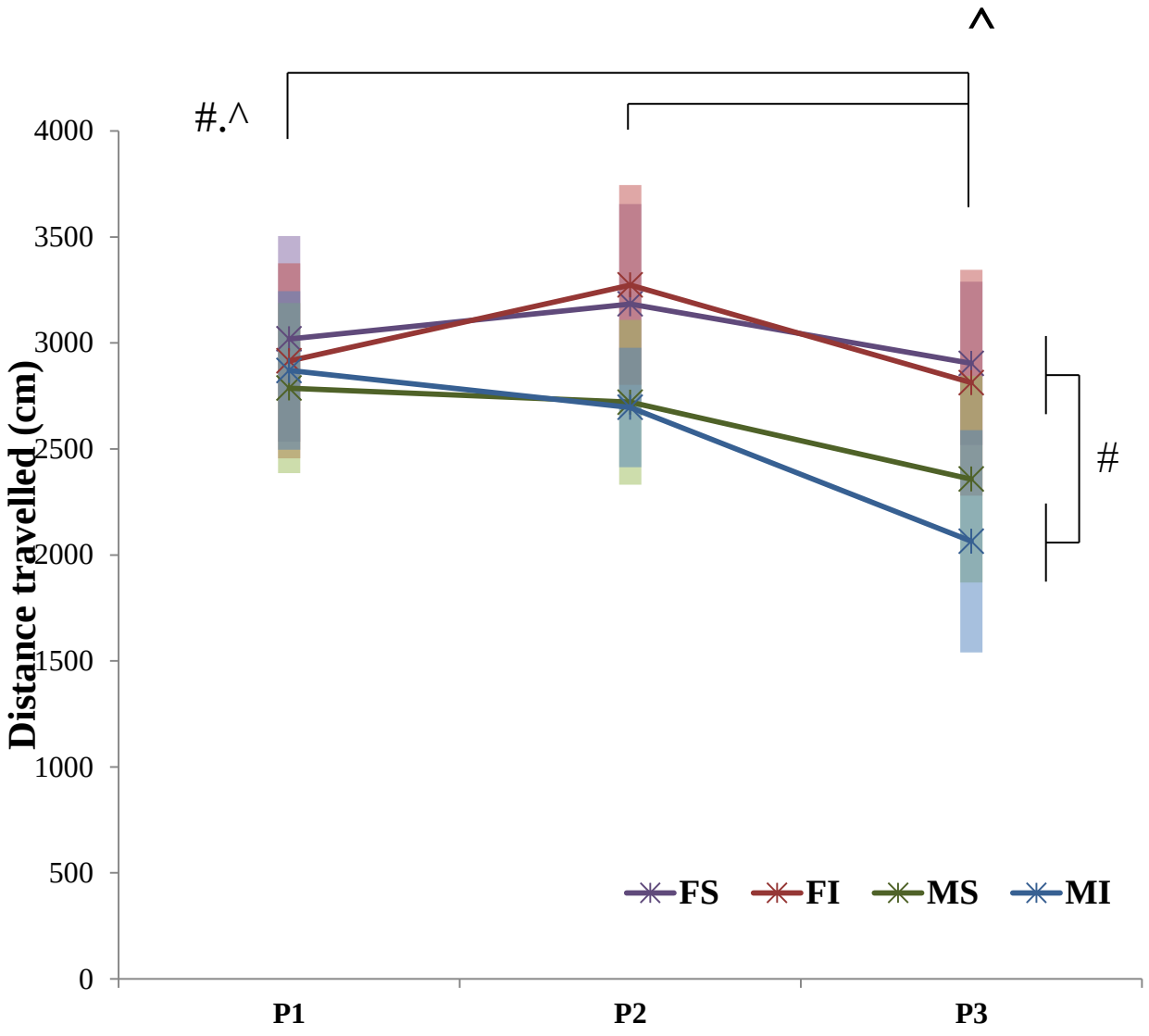

Figure 46- NOR All phases 5 MIN, Distance travelled (cm)

Distance travelled (cm) during first 5 minutes of all three phases of the NOR test for each sex and housing group; female socialised (FS n=24) and female isolated (FI n=23), male socialised (MS n=25) and male isolated (MI n=24). $^{\#}$A sex difference was found, $p<0.0001$, where females travelled further than males, $p<0.0001$. $^{\wedge}$A difference between the phases was found, $p<0.0001$, where the distance travelled in phase 1 was greater than the distance travelled in phase 3, $p<0.0001$ and where the distance travelled in phase 2 was greater than the distance travelled in phase 3, $p<0.0001$. $^{\#.\wedge}$A sex-phase interaction was found, see text for p values. Data are presented as mean ± SD

3.3 *IN-VITRO* SUPERFUSION

3.3.1 SF 1- GLUTAMATE-STIMULATED [^{3}H]NE RELEASE IN HIPPOCAMPUS AND PREFRONTAL CORTEX

The first analysis of the Superfusion 1 data compared differences in fractional release of [^{3}H]NE across three successive glutamate stimulations within one brain area as well as differences between prefrontal cortex and hippocampus tissue (n=30, 7 FS, 7 FI, 8 MS, 8 MI). A second comparison was then performed to find differences between prefrontal cortex and hippocampus tissue for each stimulation, (n=30, 7 FS, 7 FI, 8 MS, 8 MI). Parametric analyses were performed given that the primary variables (stimulation 1) were parametric. Full statistical tables can be found in appendix A.3.1.

When data from the hippocampus were analysed a difference was found between the stimulations ($F_{(2, 52)}$ = 80.94, $p<0.0001$), where fractional release of [^{3}H]NE resulting from stimulation 1 was greater than in both stimulation 2, $p<0.0001$ and stimulation 3, $p<0.0001$, (Figure 47). When data from the prefrontal cortex were analysed a difference was found between the stimulations ($F_{(2, 52)}$ = 72.89, $p<0.0001$), where fractional release of [^{3}H]NE resulting from stimulation 1 was greater than in both stimulation 2, $p<0.0001$ and stimulation 3, $p<0.0001$ (Figure 48). When data from stimulation 1 were analysed in both brain areas (Figure 49) a difference was found between the brain areas ($F_{(1, 26)}$ = 5.549, $p=0.026$), where fractional release of [^{3}H]NE in the hippocampus was greater than in the prefrontal cortex, $p=0.0222$. When data from stimulations 2 and 3 were analysed neither housing nor sex differences were found between fractional release of [^{3}H]NE in the hippocampus and prefrontal cortex (not shown graphically).

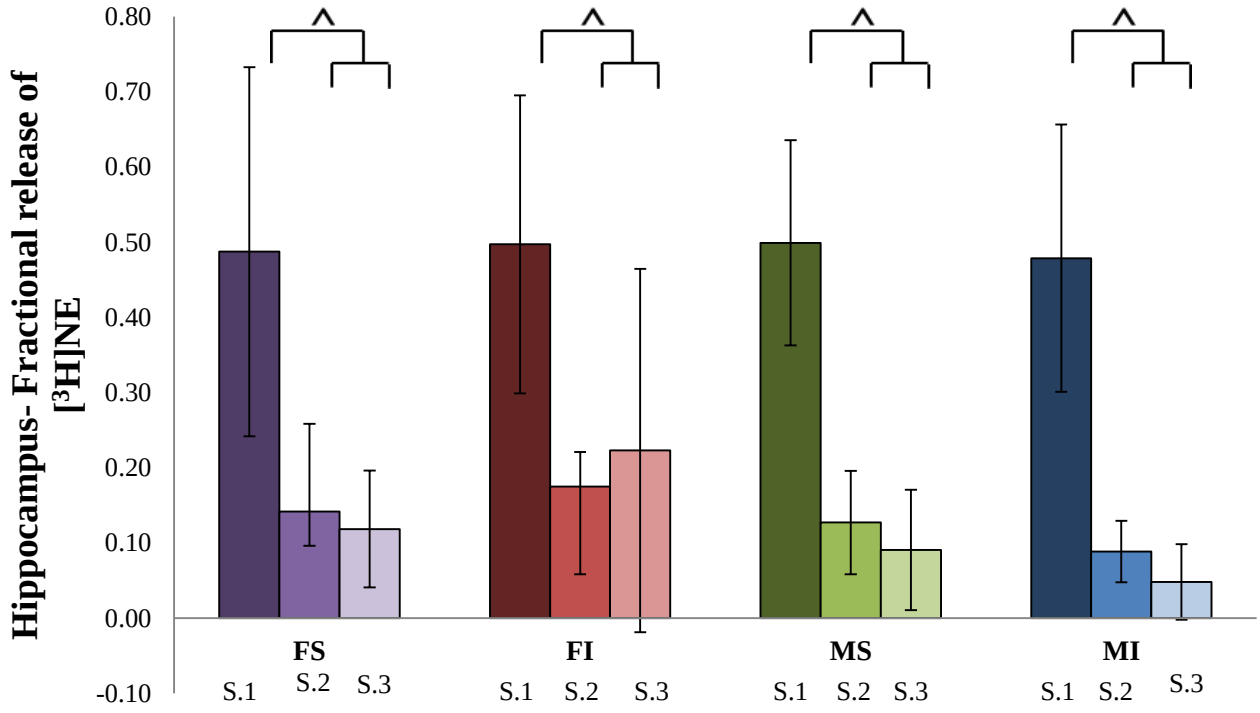

Figure 47- SF1, HC fractional release of [^{3}H]NE

Glutamate-stimulated fractional release of [^{3}H]NE in the hippocampus during Superfusion 1, for each sex and housing group; female socialised (FS n=7) and female isolated (FI n=7), male socialised (MS n=8), male isolated (MI n=8). ^ A difference was found, $p<0.0001$, where fractional release of [^{3}H]NE in S1 was greater than S2, $p<0.0001$and in S3, $p<0.0001$. Data are presented as mean ± SD. S1 = stimulation 1, S2 = stimulation 2, S3 = stimulation 3.

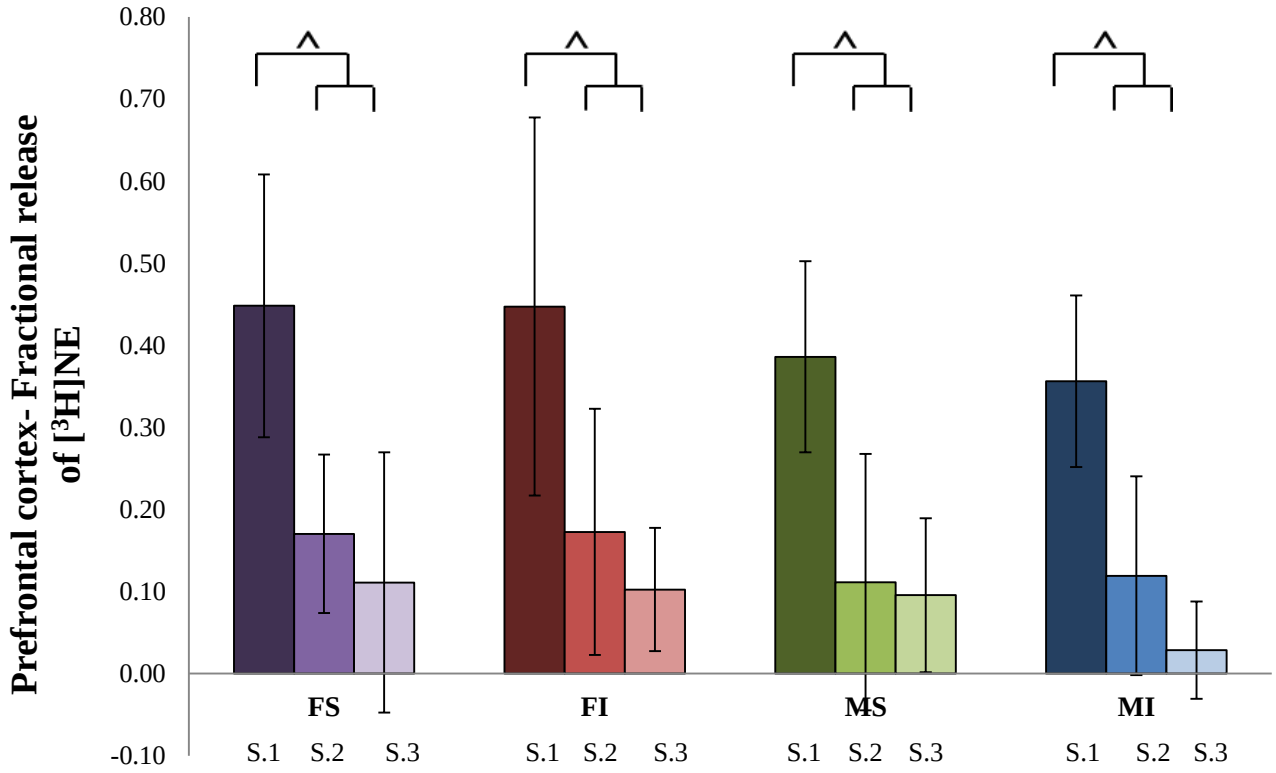

Figure 48- SF1, PFC fractional release of [3H]NE

Glutamate-stimulated fractional release of [^{3}H]NE in the prefrontal cortex during Superfusion 1, for each sex and housing group; female socialised (FS n=7) and female isolated (FI n=7), male socialised (MS n=8), male isolated (MI n=8). ^A difference was found, $p<0.0001$, where fractional release of [3H]NE in S1 was greater than S2, $p<0.0001$and in S3, $p<0.0001$. Data are presented as mean ± SD. S1 = stimulation 1, S2 = stimulation 2, S3 = stimulation 3.

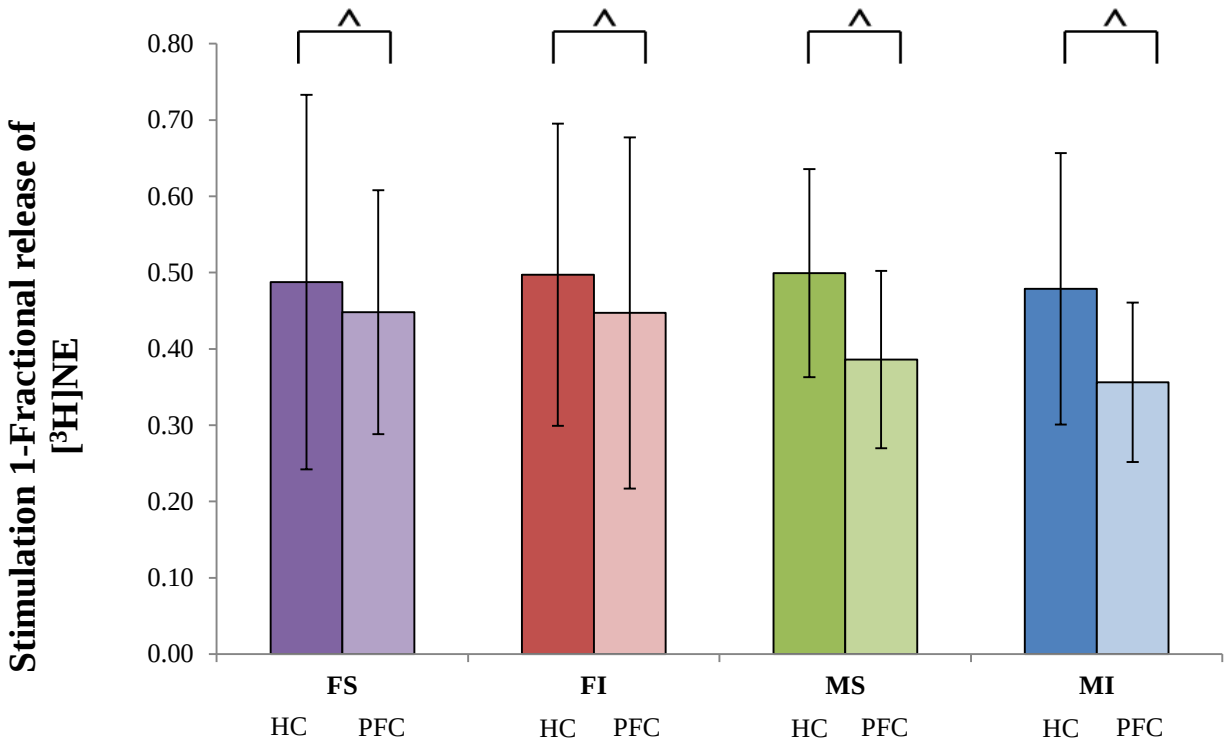

Figure 49- SF1, Stimulation 1 fractional release of [^{3}H]NE

Glutamate-stimulated fractional release of [^{3}H]NE in the hippocampus and prefrontal cortex during Superfusion 1 stimulation 1 for each sex and housing group; female socialised (FS n=7) and female isolated (FI n=7), male socialised (MS n=8), male isolated (MI n=8). ^A brain area difference was found, p=0.0263, where fractional release of [^{3}H]NE resulting from stimulation 1 was greater in the hippocampus than in the prefrontal cortex, p=0.0222. Data are presented as mean ± SD. HC= hippocampus, PFC= prefrontal cortex.

3.3.2 SF 2- GLUTAMATE-STIMULATED [^{3}H]NE RELEASE IN HIPPOCAMPUS IN PRESENCE OF MK-801 AND/OR CNQX

The first analysis of the Superfusion 2 data compared differences in glutamate-stimulated fractional release of [^{3}H]NE for each drug condition; control (glutamate alone), CNQX, MK-801 or CNQX+MK-801 across the three successive stimulations in the hippocampus. The second analysis of the Superfusion 2 data compared differences in glutamate-stimulated fractional release of [^{3}H]NE for each successive stimulation (1, 2 or 3) across the four drug conditions within the hippocampus, (n=30, FS 6, FI 6, MS 6, MI 6). Three out of the four primary variables (stimulation 1) were parametric CNQX, MK-801and CNQX-MK-901 but not control (glutamate alone), however, since this condition was identical to the control in Superfusion 1 experiment where it was parametric, parametric analyses were used for Superfusion 2. Full statistical tables can be found in appendix A.3.2.

When data from the control condition (glutamate alone,) were analysed (Figure 50), a sex difference was found ($F_{(1, 20)}$ = 4.464, p=0.0473), where fractional release of [^{3}H]NE was greater in females than in males, p=0.0473. A difference was also found between the stimulations ($F_{(2, 40)}$ = 72.45, p<0.0001), where fractional release of [^{3}H]NE resulting from stimulation 1 was greater than from stimulation 2, p<0.0001 and from stimulation 3, p<0.0001 for control data. When data from the CNQX condition were analysed (Figure 51), a sex difference was found ($F_{(1, 19)}$ = 4.395, p=0.0496), where fractional release of [^{3}H]NE was greater in females than in males, p=0.0486. A difference was also found between the stimulations ($F_{(2, 38)}$ = 60.68, p<0.0001), where fractional release of [^{3}H]NE resulting from stimulation 1 was greater than from stimulation 2, p<0.0001 and from stimulation 3, p<0.0001 and where fractional release of [^{3}H]NE resulting from stimulation 2 was greater than from stimulation 3, p=0.0298 for CNQX data. When data from the MK-801 condition were analysed (Figure 52), a difference was found between the stimulations ($F_{(2, 40)}$ = 16.97, p<0.0001), where fractional release of [^{3}H]NE resulting from stimulation 1 was greater than from stimulation 2, p=0.0004 and from stimulation 3, p<0.0001.When data from the CNQX+MK-801 condition were analysed (Figure 53), a difference was found between the stimulations ($F_{(2, 40)}$ = 69.82, p<0.0001), where fractional release of [^{3}H]NE resulting from stimulation 1 was greater than from stimulation 2, p<0.0001 and from stimulation 3, p<0.0001.When data from stimulation 1 only for all drug conditions were analysed (Figure 54), a sex difference was found ($F_{(1, 19)}$ = 8.701,

p=0.008), where fractional release of [^{3}H]NE was greater in females than in males, p=0.0060. When data from stimulations 2 and 3 were analysed neither housing nor sex differences were found between the drug conditions (not shown graphically).

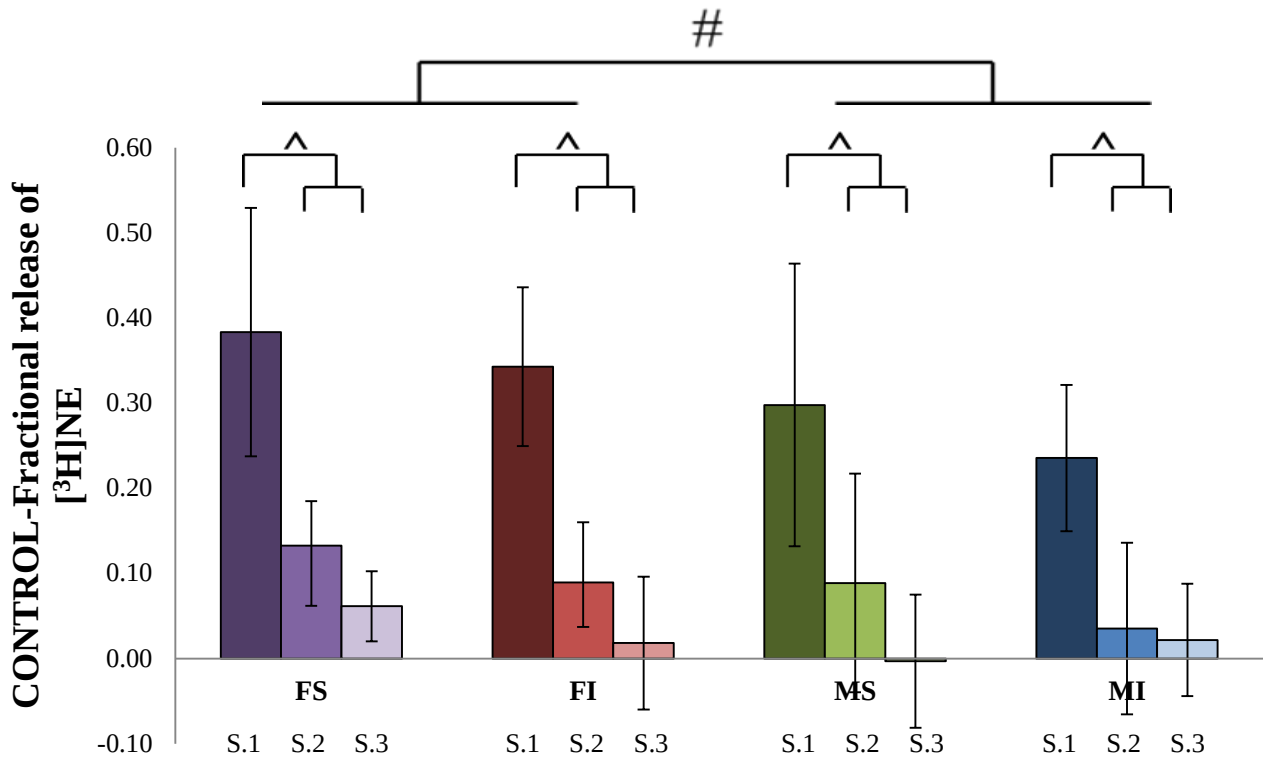

Figure 50- SF2, Control condition fractional release of [^{3}H]NE

Control condition glutamate-stimulated fractional release of [^{3}H]NE in the hippocampus during Superfusion 2, for each sex and housing group; female socialised (FS n=6) and female isolated (FI n=6), male socialised (MS n=6), male isolated (MI n=6). $^{\#}$A sex difference was found, p=0.0473, where fractional release of [^{3}H]NE in females was greater than in males, p=0.0473. $^{\wedge}$A difference was found between the stimulations p<0.0001, where fractional release of [^{3}H]NE resulting from S1 was greater than from S2, p<0.0001and from S3, p<0.0001. Data are presented as mean ± SD. S1 = stimulation 1, S2 = stimulation 2, S3 = stimulation 3.

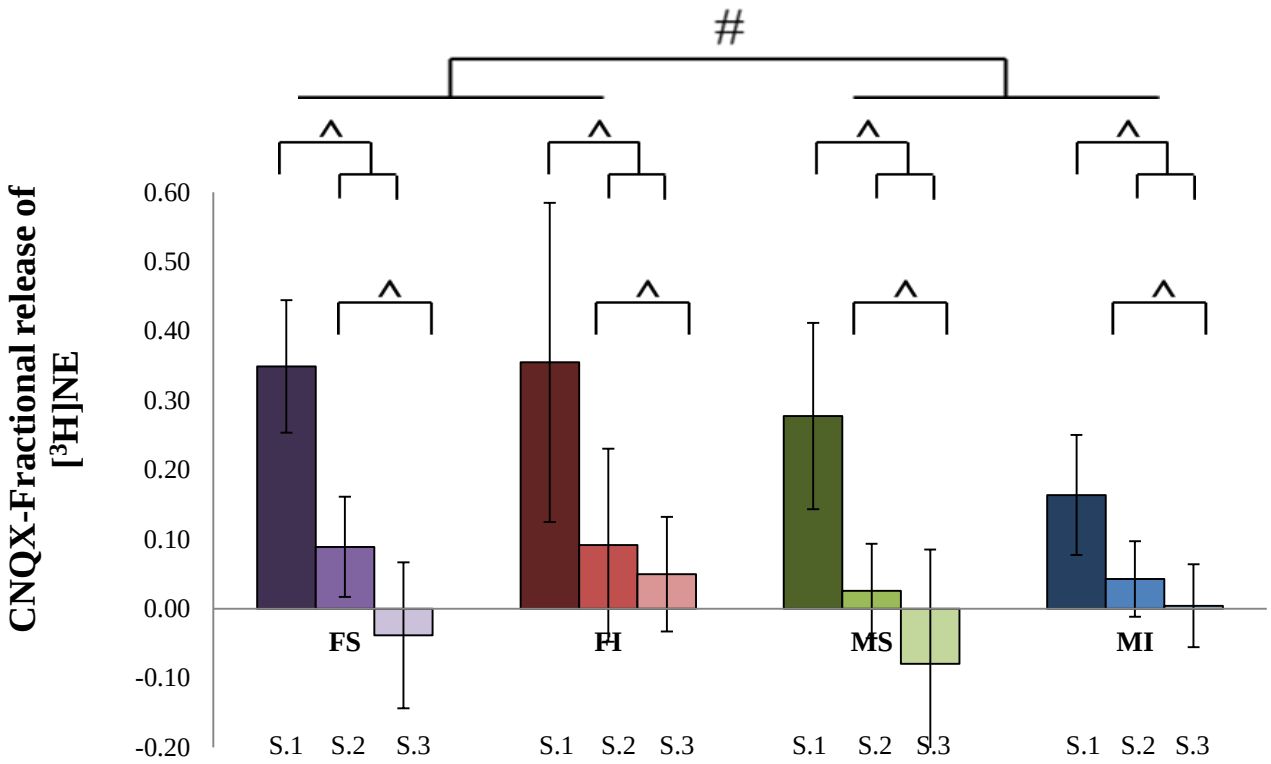

Figure 51- SF2, CNQX condition fractional release of [3H]NE

Glutamate-stimulated fractional release of [^{3}H]NE in the presence of CNQX in the hippocampus during Superfusion 2, each sex and housing group; female socialised (FS n=6) and female isolated (FI n=6), male socialised (MS n=6), male isolated (MI n=6). $^{\#}$A sex difference was found, p= 0.0496), where fractional release of [^{3}H]NE in females was greater than in males, p= 0.0486. $^{\wedge}$A difference was found between the stimulations, p<0.0001, where fractional release of [^{3}H]NE resulting from S1 was greater than from S2, p<0.0001 and from S3, p<0.0001 and fractional release of [^{3}H]NE resulting from S2 was greater than from S3, p=0.0298. Data are presented as mean ± SD. S1 = stimulation 1, S2 = stimulation 2, S3 = stimulation 3.

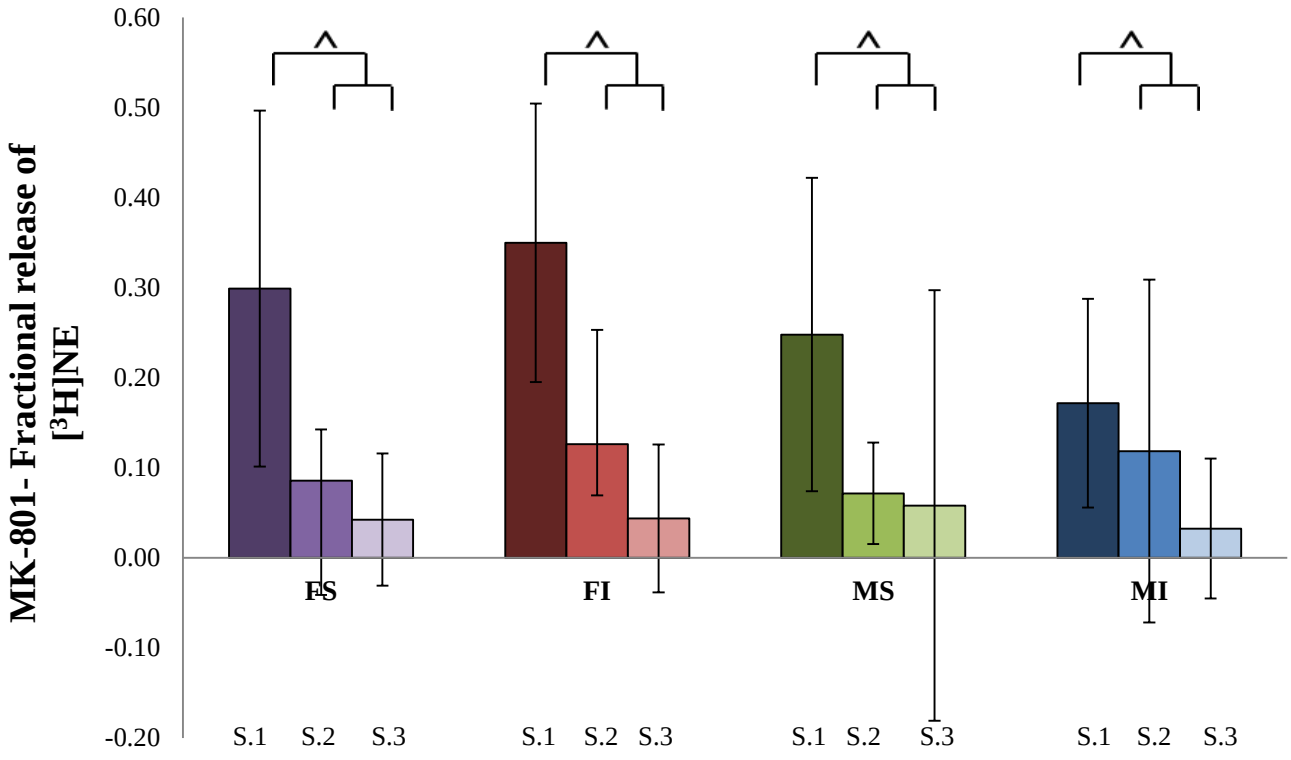

Figure 52- SF2, MK-801 condition fractional release of [3H]NE

Glutamate-stimulated fractional release of [^{3}H]NE in the presence of MK-801 in the hippocampus during Superfusion 2, for each sex and housing group; female socialised (FS n=6) and female isolated (FI n=6), male socialised (MS n=6), male isolated (MI n=6). ^A difference was found between the stimulations, $p<0.0001$, where fractional release of [^{3}H]NE resulting from S1 was greater than from S2, $p=0.0004$ and from S3, $p<0.0001$. Data are presented as mean ± SD. S1 = stimulation 1, S2 = stimulation 2, S3 = stimulation 3.

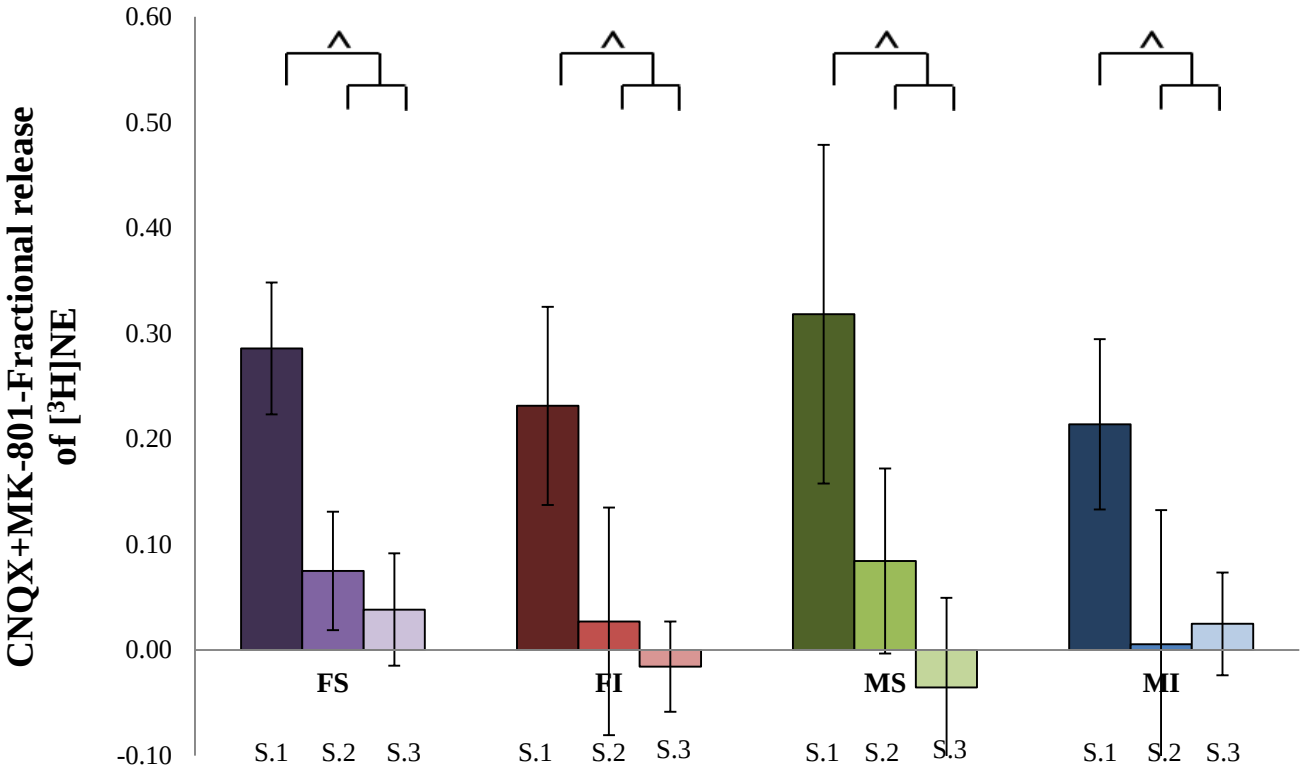

Figure 53- SF2, CNQX + MK-801 condition fractional release of [3H]NE

Glutamate-stimulated fractional release of [^{3}H]NE in the presence of CNQX and MK-801 in the hippocampus during Superfusion 2, for each sex and housing group; female socialised (FS n=6) and female isolated (FI n=6), male socialised (MS n=6), male isolated (MI n=6). ^A difference was found between the stimulations, $p<0.0001$, where fractional release of [^{3}H]NE resulting from S1 was greater than from S2, $p<0.0001$ and from S3, $p<0.0001$. Data are presented as mean ± SD. S1 = stimulation 1, S2 = stimulation 2, S3 = stimulation 3.

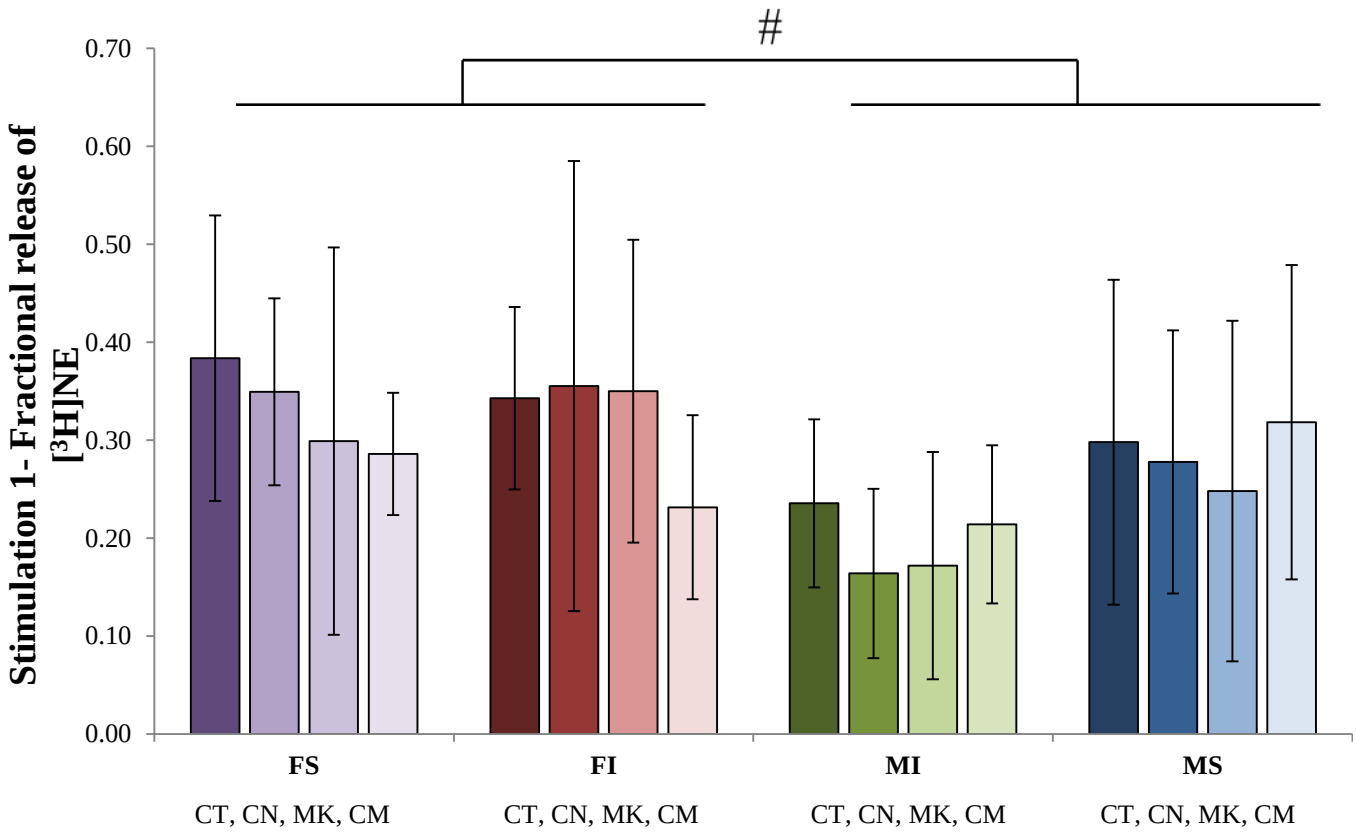

Figure 54- SF2, Stimulation 1 fractional release of [3H]NE

Glutamate-stimulated fractional release of [^{3}H]NE for all four drug conditions during stimulation 1 for each sex and housing group; female socialised (FS n=6) and female isolated (FI n=6), male socialised (MS n=6), male isolated (MI n=6). $^{\#}$A sex difference was found, p=0.008, where fractional release of [^{3}H]NE resulting from stimulation 1 was greater in females than in males, p=0.0060. Data are presented as mean ± SD. CT= control (glutamate alone), CN= CNQX, MK= MK-801, CM= CNQX+MK-801.

3.3.3 SF 3- GLUTAMATE, GABA AND KCL-STIMULATED [^{3}H]NE RELEASE IN HIPPOCAMPUS AND PREFRONTAL CORTEX

Analysis of the Superfusion 3 experimental data compared differences in fractional release of [^{3}H]NE after sequential stimulations with glutamate then GABA or vice versa. First, fractional release of [^{3}H]NE in the hippocampus was compared for the following stimulation conditions; glutamate stimulation 1, GABA stimulation 2, GABA stimulation 1 and glutamate stimulation 2 (n=33, FS 11, FI 11, MS 6, MI 5). This comparison was repeated for the corresponding data in the prefrontal cortex (n=36, FS 12, FI 12, MS 6, MI 6). Next, fractional release of [^{3}H]NE resulting from glutamate stimulation 1 was compared between the hippocampus and prefrontal cortex. Fractional release of [^{3}H]NE resulting from GABA stimulation 1 was compared between the hippocampus and prefrontal cortex. Finally, fractional release of [^{3}H]NE resulting from KCl were compared between groups for each of the 4 stimulation 3 column variables, none of these comparisons were significant (included in appendix). Therefore, the 2 KCl stimulation columns from each brain area were averaged and compared. Full statistical tables can be found in appendix A.3.3.

When fractional release of [^{3}H]NE in the hippocampus was compared for the following stimulation conditions; glutamate stimulation 1, GABA stimulation 2, GABA stimulation 1 and glutamate stimulation 2 (Figure 55), a main effect of housing was found ($F_{(1, 29)}$ = 4.738, p=0.0377) only, as the post-hoc test did not reveal specific group differences. A stimulation difference was also found for the hippocampus data ($F_{(3, 87)}$ = 3.893, p=0.0116), where fractional release of [^{3}H]NE was greater with glutamate stimulation 1 than with GABA stimulation 1, p=0.0144. When fractional release of [^{3}H]NE in the prefrontal cortex was compared for the following stimulation conditions; glutamate stimulation 1, GABA stimulation 2, GABA stimulation 1 and glutamate stimulation 2 (Figure 56), a stimulation-housing interaction was found ($F_{(3, 96)}$ = 4.161, p=0.0081) only, as the post-hoc test did not reveal specific group differences. A housing-sex-stimulation interaction was also found for prefrontal cortex data ($F_{(3, 96)}$ = 2.880, p=0.0398) only, as the post-hoc test did not reveal specific group differences. When fractional release of [^{3}H]NE resulting from glutamate stimulation 1 was compared between the hippocampus and prefrontal cortex (Figure 57), a housing-sex interaction was found ($F_{(1, 31)}$ = 5.203, p=0.0295) only, as the post-hoc test did not reveal specific group differences. A difference between the brain areas was also found for glutamate

stimulation 1 data ($F_{(1, 31)}$ = 8.050, p=0.0079), where fractional release of [^{3}H]NE was greater in the hippocampus than in the prefrontal cortex, p=0.0093. When fractional release of [^{3}H]NE resulting from GABA stimulation 1 was compared between the hippocampus and prefrontal cortex (Figure 58), a housing-brain area interaction was found ($F_{(1, 29)}$ = 5.625, p=0.0245) only, as the post-hoc test did not reveal specific group differences. When fractional release of [^{3}H]NE resulting from KCl stimulation 3 was compared between the hippocampus and prefrontal cortex (Figure 59), a sex difference was found ($F_{(1, 31)}$ = 7.685, p=0.0093), where release in males was greater than in females, p=0.0104. A difference between the brain areas was also found ($F_{(1, 31)}$ = 9.715, p=0.0039), where KCl-stimulated release of [^{3}H]NE was greater in the prefrontal cortex than in the hippocampus, p=0.0042.

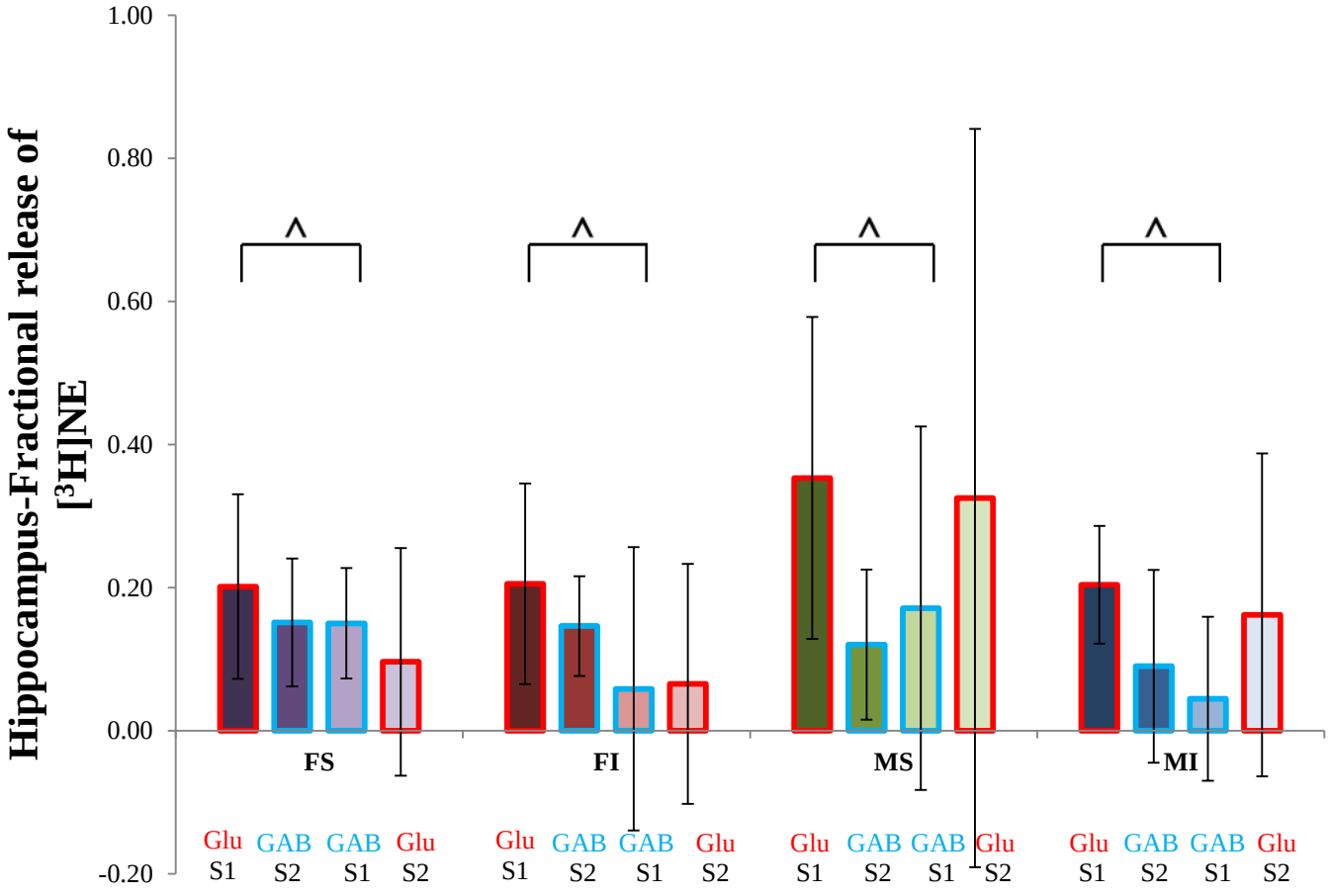

Figure 55- SF3, HC fractional release of [3H]NE

Glutamate and GABA stimulated fractional release of [^{3}H]NE in the hippocampus during Superfusion 3 stimulations 1 and 2, for each sex and housing group; female socialised (FS n=12) and female isolated (FI n=12), male socialised (MS n=5), male isolated (MI n=6). A main effect of

housing was found, p=0.0377, this effect was not supported in post hoc testing. ^A stimulation difference was found, p=0.0116, where fractional release of [^{3}H]NE resulting from glutamate stimulation 1 was greater than from GABA stimulation 1, p=0.0144. Data are presented as mean ± SD. S1 = stimulation 1, S2 = stimulation 2, Glu = glutamate, GAB = GABA.

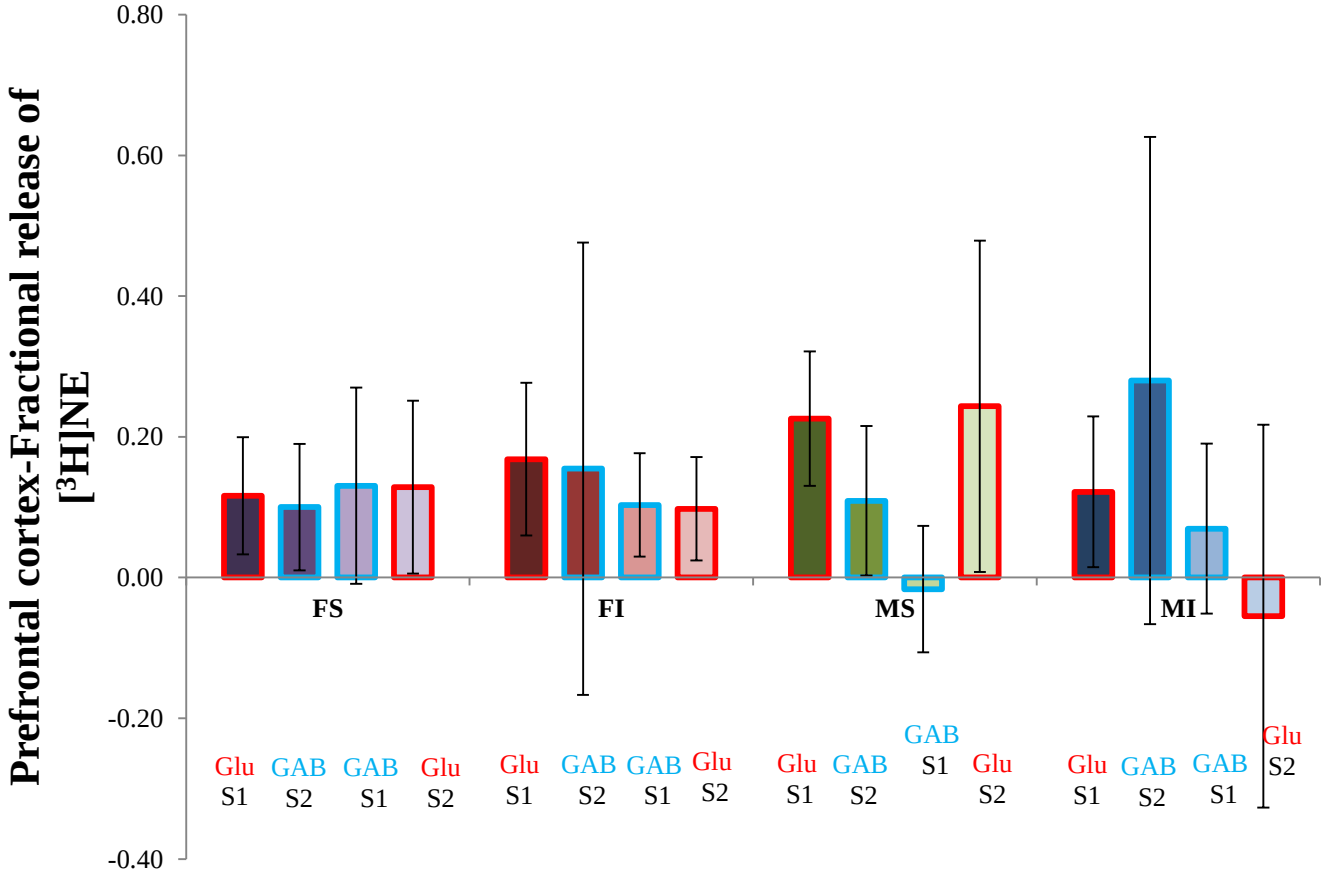

Figure 56- SF3, PFC fractional release of [3H]NE

Glutamate and GABA stimulated fractional release of [^{3}H]NE in the prefrontal during Superfusion 3 stimulations 1 and 2, for each sex and housing group; female socialised (FS n=12) and female isolated (FI n=12), male socialised (MS n=5), male isolated (MI n=6). A housing-stimulation interaction was found, p=0.0081, this interaction was not supported in post hoc testing. A housing-sex-stimulation interaction was found p=0.0398, this interaction was not supported in post hoc testing. Data are presented as mean ± SD. S1 = stimulation 1, S2 = stimulation 2, Glu = glutamate, GAB = GABA.

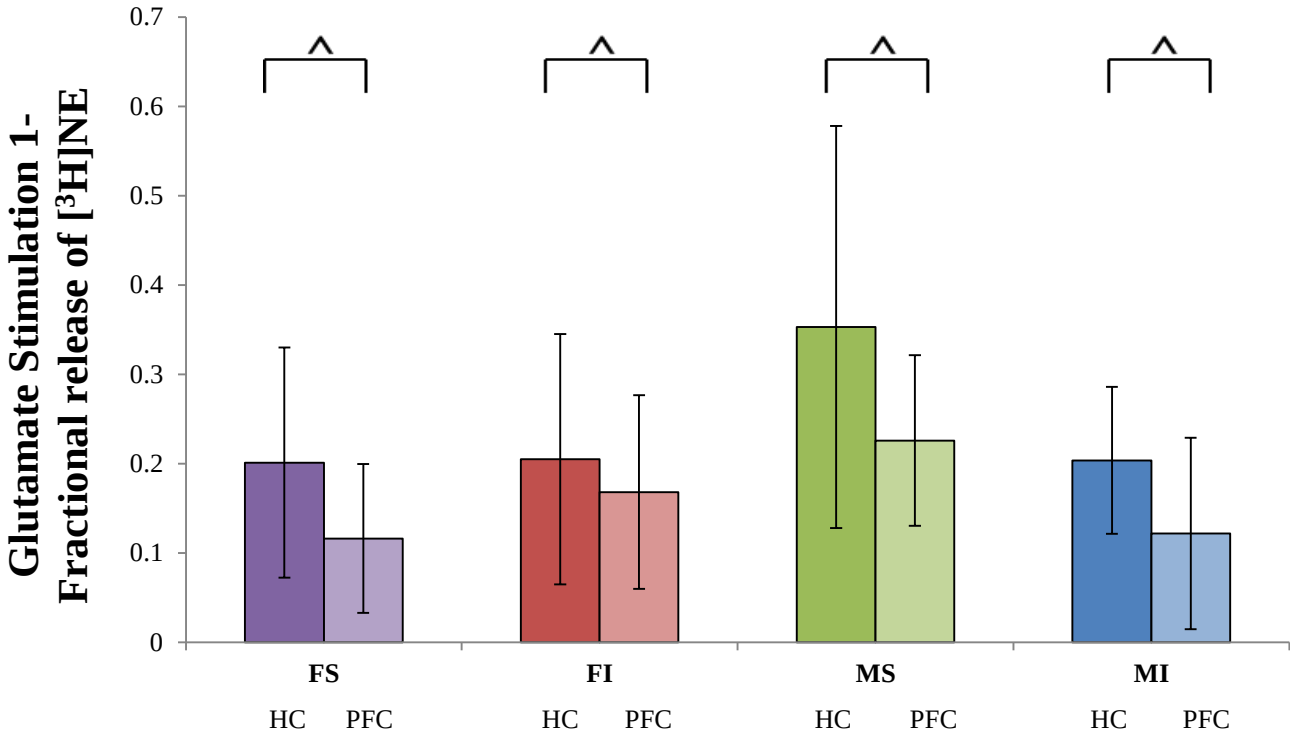

Figure 57- SF3, Glutamate stimulation 1 fractional release of [3H]NE

Glutamate-stimulated fractional release of [^{3}H]NE in the hippocampus and prefrontal cortex during Superfusion 3 stimulation 1, for each sex and housing group; female socialised (FS n=12) and female isolated (FI n=12), male socialised (MS n=5), male isolated (MI n=6). ^A difference was found between the brain areas, p=0.0079, where fractional release of [^{3}H]NE was greater in the hippocampus than in the prefrontal cortex, p=0.0093. A housing-sex interaction was found, p=0.0295, this interaction was not supported in post hoc testing. Data are presented as mean ± SD. HC = hippocampus, PFC = prefrontal cortex.

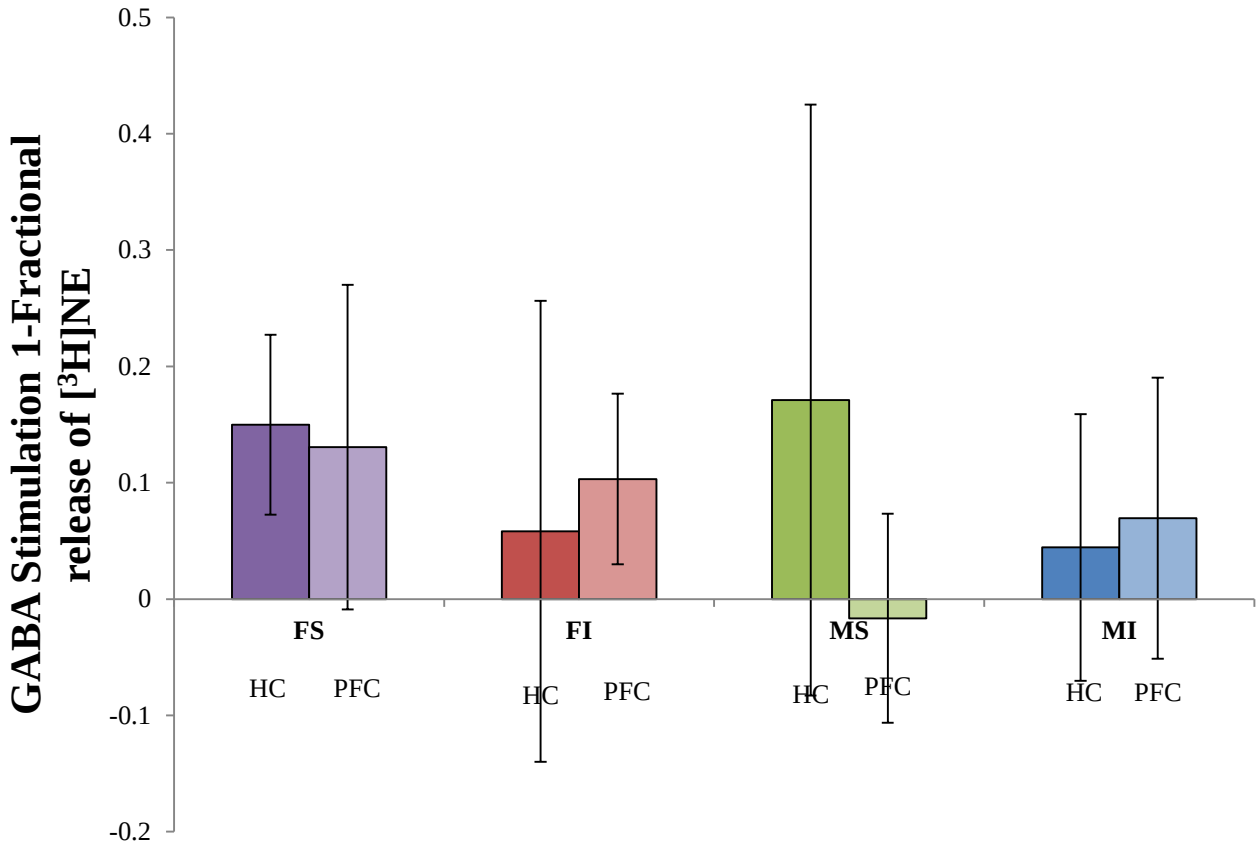

Figure 58- SF3, GABA stimulation 1 fractional release of [3H]NE

GABA-stimulated fractional release of [^{3}H]NE in the hippocampus and prefrontal cortex during Superfusion 3 stimulation 1, for each sex and housing group; female socialised (FS n=12) and female isolated (FI n=12), male socialised (MS n=5), male isolated (MI n=6). A housing-brain area interaction was found, p=0.0245, this interaction was not supported in post hoc testing. Data are presented as mean ± SD. HC = hippocampus, PFC = prefrontal cortex.

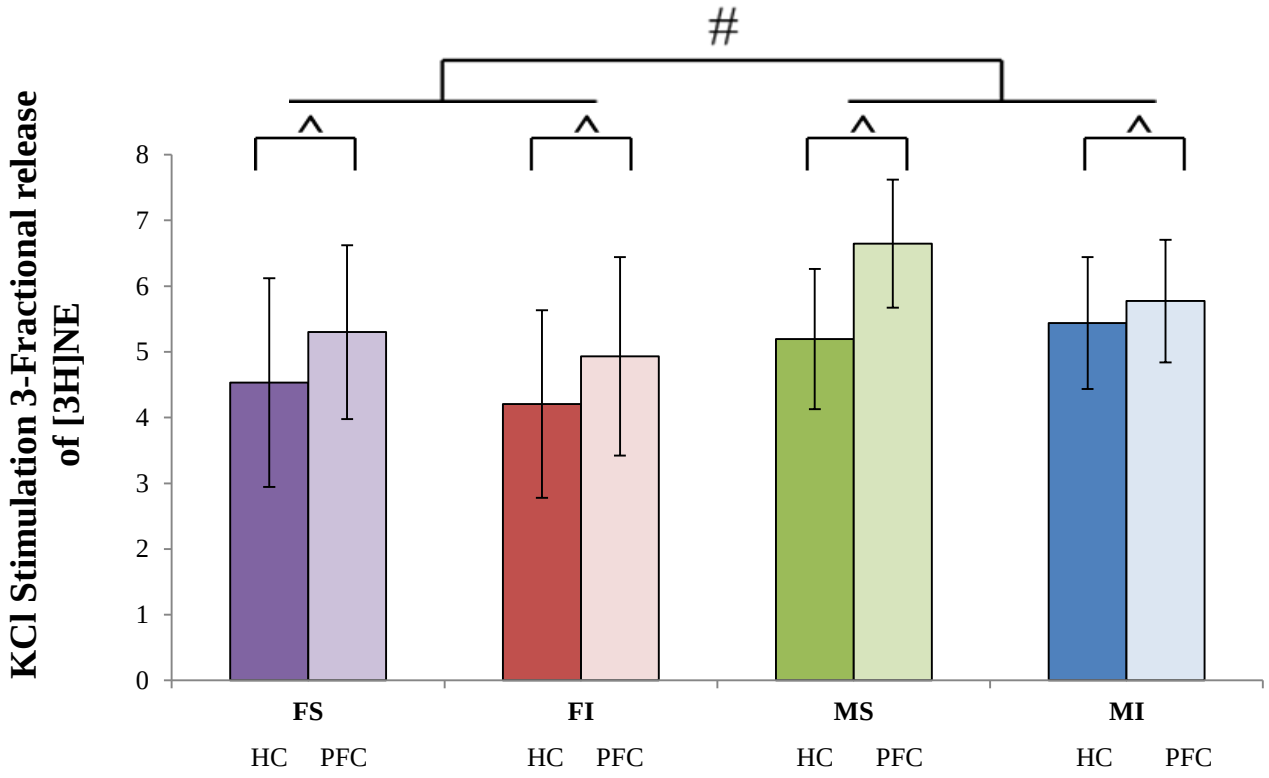

Figure 59- SF3, KCl stimulation 3 fractional release of [^{3}H]NE

KCl-stimulated fractional release of [^{3}H]NE in the hippocampus and prefrontal cortex during Superfusion 3 stimulation 3, for each sex and housing group; female socialised (FS n=12) and female isolated (FI n=12), male socialised (MS n=6), male isolated (MI n=6). A sex difference was found, p=0.0093, where release in males was greater than in females, p=0.0104. A difference between the brain areas was found, p=0.0039, where KCl-stimulated release of [^{3}H]NE was greater in the prefrontal cortex than in the hippocampus, p=0.0042.

3.4 ELISA AND BCA PROTEIN ASSAY

ELISAs were performed on rat brain tissue homogenates, hippocampus and prefrontal cortex, to quantify concentrations of neurotransmitter, NE (n=24, FS 6, FI 6, MS 6, MI 6) and glutamate (n=40, FS 10, FI 10, MS 10, MI 10), these were expressed as ng or mg/g wet weight of tissue respectively. BCA protein assays were performed from homogenates made for ELISA experiments to quantify concentrations of total protein in the tissue. ELISA values were divided by BCA protein assay values to give a value for neurotransmitter expressed as pg or mg /g total protein. Full statistical tables can be found in appendix A.4.

When data were analysed for the NE concentration in wet weight of tissue (NE ng/g) in the hippocampus (Figure 60), a sex difference was found ($F_{(1,\ 20)}$ = 4.745, p=0.0415), where the concentration of NE was higher in females than in males, p=0.0415. When data were analysed for the NE concentration in the total protein (NE pg/g) in the hippocampus (Figure 61), a sex difference was found ($H_{(3,\ N=39)}$ = 7.854, p=0.0491), where the concentration of NE was higher in FS than in MS, p=0.0362. When data were analysed for the NE concentration in wet weight of tissue (NE ng/g) in the prefrontal cortex (Figure 62), a sex difference was found ($F_{(1,\ 20)}$ = 5.132, p=0.0347), where the concentration of NE was higher in males than in females, p=0.0347. Neither housing nor sex differences were found when data were analysed for the NE concentration in the total protein (NE pg/g) in the prefrontal cortex (Figure 63). Neither housing nor sex differences were found when data were analysed for the glutamate concentration in wet weight of tissue (Glu mg/g) in the hippocampus (Figure 64). Neither housing nor sex differences were found when data were analysed for the glutamate concentration in the total protein (Glu mg/g) in the hippocampus (Figure 65). Neither housing nor sex differences were found when data were analysed for the glutamate concentration in wet weight of tissue (Glu mg/g) in the prefrontal cortex (Figure 66).

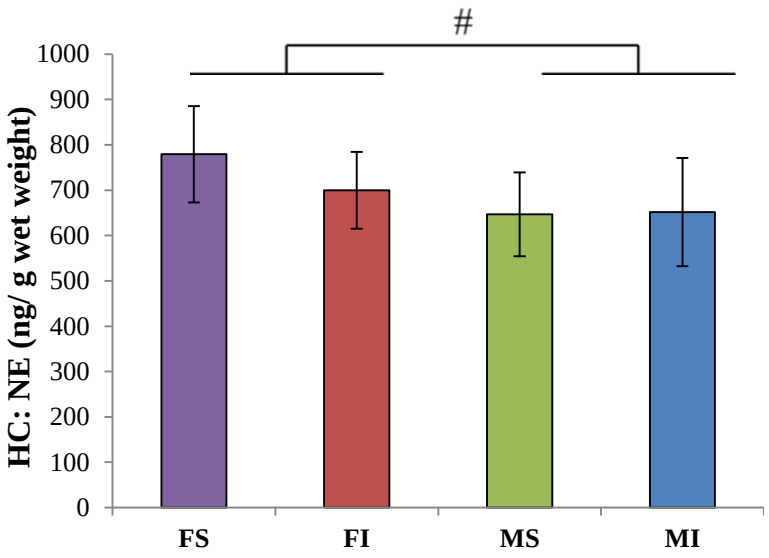

Figure 60- ELISA, HC NE (ng/g wet weight)

The concentration of norepinephrine (NE ng/g wet weight of tissue) in the hippocampus was analysed for each sex and housing group; female socialised (FS n=6) and female isolated (FI n=6), male socialised (MS n=6), male isolated (MI n=6). #A sex difference was found, p=0.0415, where the concentration of NE was higher in females than in males, p=0.0415. Data are presented as mean ± SD.

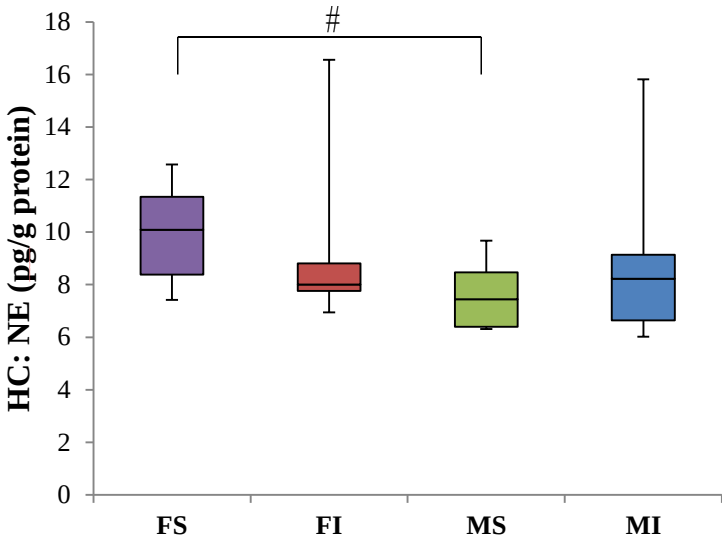

Figure 61- ELISA BCA assay, HC NE (pg/g protein)

The concentration of norepinephrine (NE pg/g protein) in the hippocampus was analysed for each sex_housing group; female socialised (FS n=10) and female isolated (FI n=10), male socialised (MS n=10), male isolated (MI n=10). #A sex difference was found, p=0.0491, where the concentration of

NE was higher in FS than in MS, p=0.0362. Data are presented as median ± IQR with min and max values.

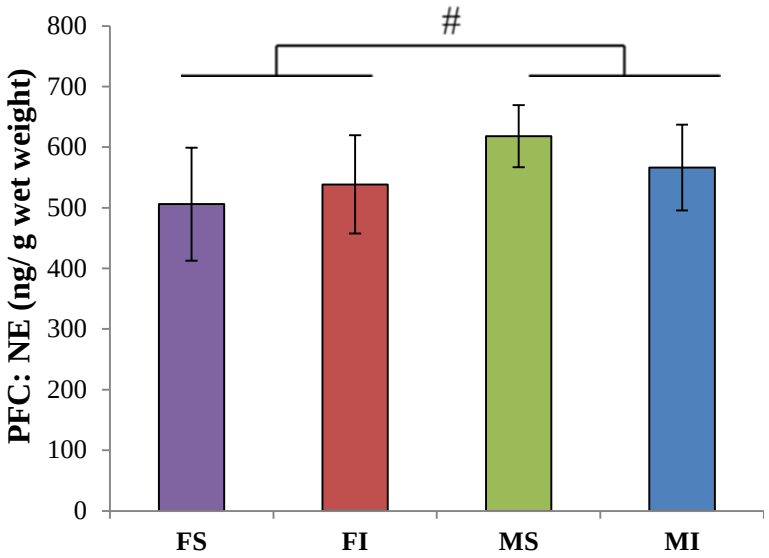

Figure 62- ELISA, PFC NE (ng/g wet weight)

The concentration of norepinephrine (NE ng/g wet weight of tissue) in the prefrontal cortex was analysed for each sex and housing group; female socialised (FS n=6) and female isolated (FI n=6), male socialised (MS n=6), male isolated (MI n=6). #A sex difference was found, p=0.0347, where the concentration of NE was higher in males than in females, p=0.0347. Data are presented as mean ± SD.

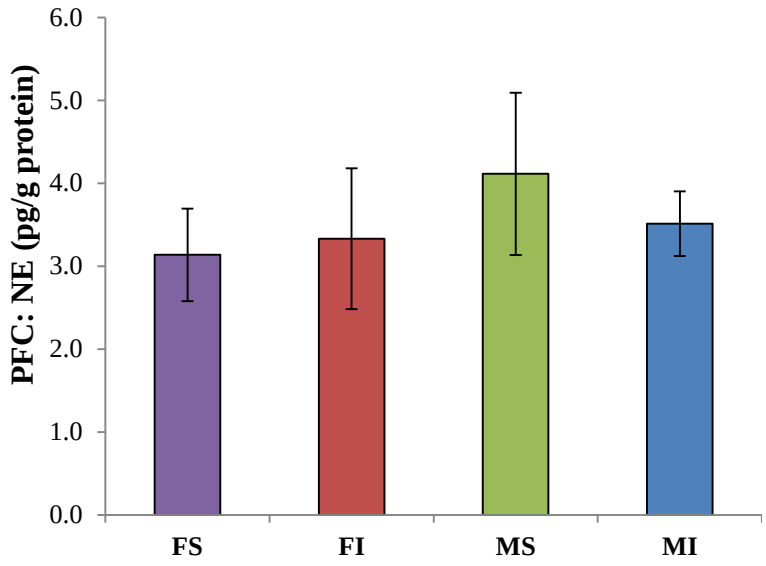

Figure 63- ELISA BCA assay, PFC NE (pg/g protein)

The concentration of norepinephrine (NE pg/g protein) in the prefrontal cortex was analysed for each sex and housing group; female socialised (FS n=6) and female isolated (FI n=6), male socialised (MS n=6), male isolated (MI n=6). Neither housing nor sex differences were found. Data are presented as mean ± SD.

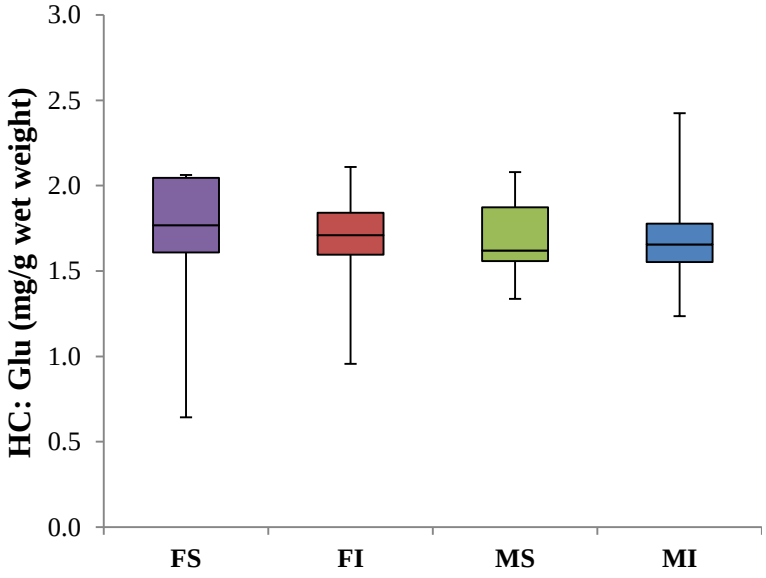

Figure 64- ELISA, HC Glu (mg/g wet weight)

The concentration of glutamate (Glu mg/g wet weight of tissue) in the hippocampus was analysed for each sex and housing group; female socialised (FS n=10) and female isolated (FI n=10), male socialised (MS n=10), male isolated (MI n=10). Neither housing nor sex differences were found. Data are presented as median ± IQR with min and max values.

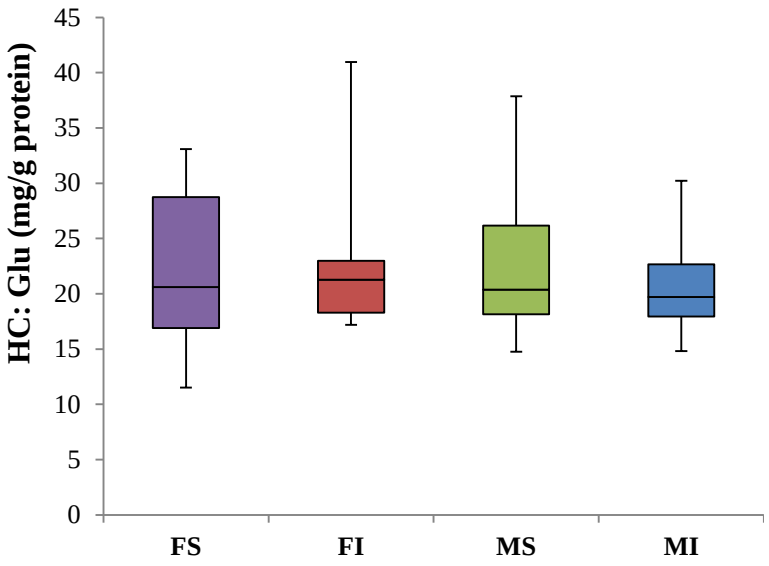

Figure 65- ELISA BCA assay, HC Glu (mg/g protein)

The concentration of glutamate (Glu mg/g protein) in the hippocampus was analysed for each sex and housing group; female socialised (FS n=10) and female isolated (FI n=10), male socialised (MS n=10), male isolated (MI n=10). Neither housing nor sex differences were found. Data are presented as median ± IQR with min and max values.

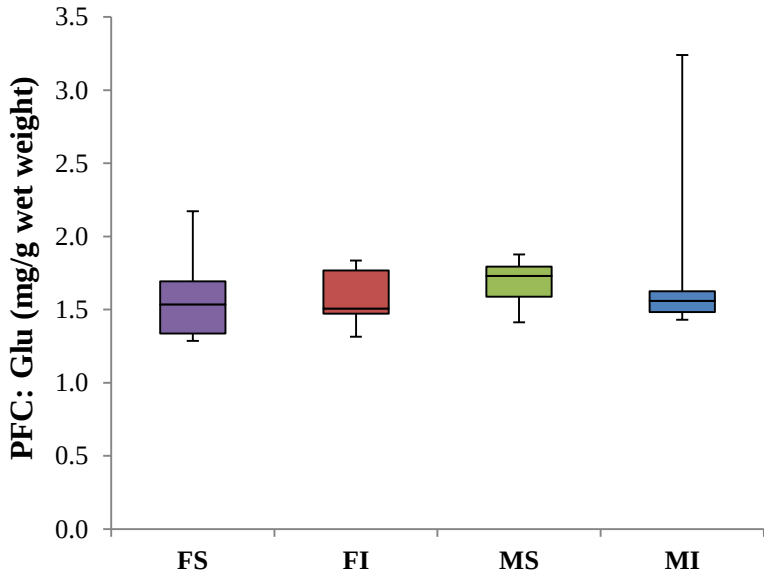

Figure 66- ELISA, PFC Glu (mg/g wet weight)

The concentration of glutamate (Glu mg/g wet weight of tissue) in the prefrontal cortex was analysed for each sex and housing group; female socialised (FS n=6) and female isolated (FI n=6), male socialised (MS n=6), male isolated (MI n=6). Neither housing nor sex differences were found. Data are presented as median ± IQR with min and max values.

3.5 ULTRASONIC VOCALISATIONS

Data were corrected to ensure results from the two bat detectors were comparable, see methodology and appendix for details, (n=41, 14 FS, 16 FI, 5 MS, 6 MI). When corrected mean call duration (s) and total call data were analysed neither housing nor sex differences were found (Figure 67), (Figure 68). Full statistical tables can be found in appendix A.5.

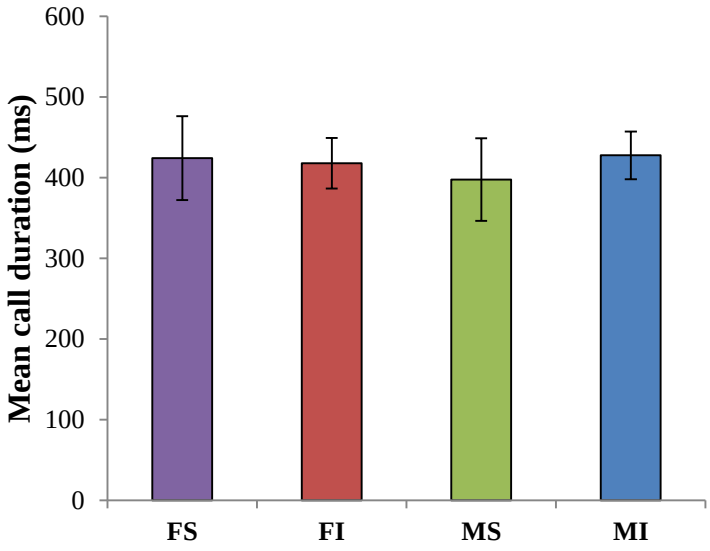

Figure 67- USV, Mean call duration (ms)

Mean call durations (ms) during USV recordings for each sex and housing group; female socialised (FS n=14) and female isolated (FI n=16), male socialised (MS n=5), male isolated (MI n=6). Neither housing nor sex differences were found. Data were corrected; see USV methodology section 2.4.2 for details. Data are presented as mean ± SD.

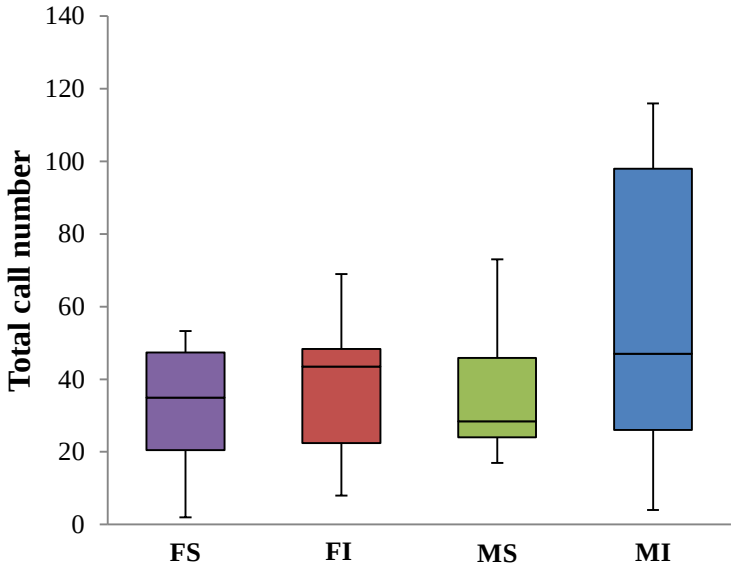

Figure 68- USV, Total call number

Total call number during USV recordings for each sex_housing group; female socialised (FS n=14) and female isolated (FI n=16), male socialised (MS n=5), male isolated (MI n=6). Neither housing nor sex differences were found. Data were corrected; see USV methodology section 2.4.2 for details. Data are presented as median ± IQR with min and max values.

CHAPTER 4
-DISCUSSION-

The primary purpose of this study was to investigate the cognitive symptoms of schizophrenia with particular focus on the attentional system. The experiments were designed to reveal behavioural deficits, specifically attentional dysfunction, induced by SIR model of schizophrenia and then to probe how aberrations in related neurochemical system, the locus-coeruleus norepinephrine system, might underlie attentional abnormalities. A secondary aim was to investigate negative symptomology tested social function resulting from SIR. Analyses were conducted to determine whether social or isolated housing differentially affected the behavioural and neurochemical tests applied. These analyses also investigated how SIR might affect males and females differently. This discussion will follow the structure of the results, first addressing all behavioural and neurochemical tests for attention and then move onto social function. In each section any housing differences found will be discussed and this will be followed by a discussion on sex differences found.

The only physical parameter recorded, body weight, was found to differ by housing and sex, this was found to compliment the literature in part. In the current study differences between the housing groups became apparent from the fourth week (p42-49) after the implementation of experimental housing conditions. At this time point isolated males weighed more than socialised males. The divergence of median body weights of the male groups was continued to the end of testing though it was not statistically significant. Isolation housing in females did not result in any significant differences in body weight. Sex differences were also found from week p42-49 such that socialised males were heavier than socialised females and isolated males were heavier than isolated females, these results were sustained for all weeks subsequent until the end point. A difference in the body weight of male and female rats is a normal physiological phenomenon which is independent to SIR (Ferreira, Foley et al. 2012).

An increase in the body weight of isolated male rats has been previously documented after around 4 weeks of SIR experimental conditioning (Sahakian, Burdess et al. 1982, Menich and Baron 1984, Weintraub, Singaravelu et al. 2010, Nakhate, Kokare et al. 2011). One such study found that isolated male S-Ds weighed more than socialised rats, as early as the second week after the

implementation of experimental housing conditions and this difference was sustained for the remaining 7 weeks of the study (Simpson and Kelly 2012). One of the key differences was that in this study the animals were placed in experimental housing from 6 weeks old as opposed to 3 weeks old, as applied in the present study. This result reinforces the capability of the model to impact a change in body weight due to housing conditions even when the conditions are implemented later in life. The literature suggests that the increased body weight of isolated males results from lack of stimulation leading to overeating in the absence of playmates coupled with lower levels of activity (Menich and Baron 1984, Simpson and Kelly 2012). Other studies have failed to find housing differences for body weight (Hellemans, Benge et al. 2004, Weiss, Pryce et al. 2004). An example of one of these studies found that there were no differences when comparing the body weights of minimally handled socialised male S-Ds to isolated male rats. However isolates did weigh more if they had been exposed to an additional stressor (maternal separation) (Ryu, Yoo et al. 2009).

In the present study, there were no housing differences evident between the body weights of the female groups. A single SIR study reported housing differences in female S-Ds (Jahng, Yoo et al. 2012), where isolates weighed more at p49 after 4 weeks of experimental conditioning. Much of the methodology of this study was consistent with the present study. The reason that the isolated females in the present study did not display increased body weight may have been due to the presence of males in the housing facility. The presence of male vocalisation and olfactory cues may have induced stress in the female rats which impacted their eating habits. It has been shown that stressed female S-Ds gain less weight than non-stressed females (Bowman, Ferguson et al. 2002).

Overall the results for body weight during experimental conditioning in the present study are fairly consistent with existing SIR literature. Male isolates were found to show differences in their weight from the fourth week of isolation, this result was reflected in existing literature; however this difference did not remain statistically significant throughout experimental conditioning. When comparing our female isolates no differences were found, this was in contrast to the only paper found which addressed the effects of isolation on female body weight. This lack of difference in females and limited differences in male may be related to the different experimental housing conditions.

4.1 NOVEL OBJECT RECOGNITION TESTING

Analysis of the NOR test was performed on data from the first minute and cumulative five minutes of each of the three phases, open-field exploration, object familiarization, and novel object recognition. In addition and only for phase 1, the open-field exploration, a cumulative ten minute analysis was performed, as this phase ran for 10 minutes while phase 2 and phase 3 only ran for 5 minutes. Analysis of the first minute provides insight to the immediate reaction to novelty whilst analysis of the total five minutes provides an insight into familiarisation to this novelty and thus attention (Brenes, Padilla et al. 2009). For the purpose of this discussion, the results from the first minute and five minutes of data from each phase will be compared in order to explore how behavioural response changed with time. Results obtained for the distance travelled (cm), the only variable recorded throughout all phases of testing, will be discussed for each individual phase and then compared across all three phases.

4.1.1 PHASE 1- OPEN FIELD EXPLORATION

The first phase of the NOR test consisted placing each rat in an open field and recording their movement around this space for 10 minutes. This first phase of testing represents the first time the animals were outside of their experimental housing conditions. It therefore provides critical information about the animals' response to a truly novel situation. Locomotor activity was compared between groups as well as entries to, and time spent within the centralised 'inner-zone' of the open field to provide a measure of anxiety-like behaviour (Das, Barhwal et al. 2015). Three temporal analyses were performed (first minute, first cumulative five minutes and full ten minutes).

One housing difference was found during this phase where socialised rats travelled further in the open field during the first minute of testing. A number of sex differences were also found; in the first minute females were hyperactive and made fewer entries to the inner-zone. In the first five minutes and full ten minutes, females similarly made fewer entries to the inner-zone. During the full ten minutes males were found to spend longer in the inner-zone.

During the first minute analysis a housing difference was evident for the distance travelled demonstrating that socialised animals travelled a greater distance than isolated animals. There is

little in the literature to support this finding as most studies analyse longer-term locomotor activity (at least five minutes) in the open field. In one study a minute-by-minute analysis was performed on 10 minutes of open field data (Brenes, Padilla et al. 2009). Briefly, male S-Ds were isolated from p28-60. At p60 the S-Ds underwent elevated plus maze testing and at p62 animals underwent open field testing. The minute analysis of the open field test revealed the following; isolated animals and socialised animals were found to travel similar differences at one minute, four minute and six minute time bins and for all other time points isolates were found to be hyperactive as well as in the overall ten minute analysis. The fact that rats underwent a behavioural test prior to open field means that subsequent measurements in the open field are not a true representation of the first time outside of the home cage, i.e. not their first experience of a novel environment. It may have been the case that the isolates would have demonstrated freezing behaviour similar to the results of the present study if they had not been exposed previously to a novel environment and subjected to less handling. Additionally the isolation period of the S-Ds was half as long as in the present study which may also have contributed to the different results of the groups. Another minute-by-minute analysis of locomotor activity during the NOR test, this time in Lister Hooded and Wistar, male and female rats revealed that there were there were no differences in exploratory activity in the first minute (Ennaceur, Michalikova et al. 2005), though these strains have been shown to have different activity levels to S-Ds (Weiss, Di Iorio et al. 2000). In the aforementioned study all animals were housed in groups of three, were handled once a day and underwent an anxiety test prior to NOR testing. Therefore our study presents a novel finding with regards to the first minute locomotor activity analysis. This fact that isolates covered a shorter distance implies that there was initial (very short term) enhanced innate freezing response. Freezing responses are used as an indicator of fear (Yusufishaq and Rosenkranz 2013). Previous work in the SIR model has used freezing responses to investigate conditioned fear as opposed to innate fear. It has been shown that isolated rats have a decreased freezing response following fear conditioning which is in contrast to the increased innate freezing response of the present study. Though this may seem conflicting, taken together these results imply abnormal attribution of salience at both ends of the spectrum which fits with the heterogeneity of symptoms in schizophrenia.

After the initial reduction in locomotor activity during the first minute of the first phase no further differences were found in the five or ten minute analyses. This implies that after initial exposure (first minute), isolates quickly went on to travel similar distances to the socialised animals. In previous SIR studies, the most commonly reported locomotor activity finding is that isolates are hyperactive compared to socialised animals and this result is used to exemplify abnormal motor

activity associated with schizophrenia (Hall 1998, Lapiz, Fulford et al. 2003, Fone and Porkess 2008). Activity is commonly measured as distance travelled or number of crossings between areas in the open field over a period of at least 10 minutes. The present study found no evidence of isolation-induced hyperactivity during a 10 minute, 5 minute or 1 minute analysis of the distance travelled in the novel arena. This does however, fit with previous findings from S-D SIR studies where hyperactivity is less-robustly induced than in other strains (Table 2). A study utilising a similar SIR methodology also found no differences in locomotion in S-D males over a 5 minute time course (Simpson and Kelly 2012). Many studies reporting hyperactivity used male Lister Hooded rats (Jones, Brown et al. 2011, Watson, Marsden et al. 2012, Zamberletti, Viganò et al. 2012). Lister Hooded rats are reported as producing more reliable changes in locomotor activity than S-D rats (Weiss, Di Iorio et al. 2000). Whilst sex and strain can create a bias in reporting SIR hyperactivity certain other methodological factors also determine the result. Critically, in the context of this this study, the time over which the recording takes place is of interest as most studies are conducted over longer durations, i.e. do not address the first minute of activity in the open-field.

In the present study when activity within the inner-zone was tested no differences were found between the housing groups. A previous study found that isolated male S-Ds made significantly fewer entries to the open field inner zone and also spent significantly less time in the inner-zone (Das, Barhwal et al. 2015). In this study isolated animals were housed in isolation chambers so that they were not exposed to any sensory cues from other animals, unlike in the present study where all rats were housed in the same room in open-topped cages. This may have led to a more robust induction of anxiety which was reflected in the open field test. It would be pertinent to test the latency to the first entry into the inner-zone. However, in the present study, the animal was placed in the centre of the arena (IZ) by the experimenter at the start of testing, so performing a statistical analysis on this would not have been valid. Previous studies have repeatedly demonstrated a slower emergence of isolates into an open area (Arakawa 2005).

With regard to the sex differences evident in the open field test. Females were hyperactive compared to males in the first minute but not during five minute and ten minute analyses. This female hyperactivity has been previously demonstrated in S-Ds in the SIR model for the first three minutes of an open field trial and in the subsequent three minutes of the trial the locomotor activity of the males and females equalised (Beck and Luine 2002). This is in agreement with the finding of the present study, as female hyperactivity is a common finding in control groups from other studies. Open field recordings lasting 6 minutes in S-Ds revealed female hyperactivity (Dubovický,

Skultétyová et al. 1999) and also showed that females take longer to habituate to new environments. Another study showed female hyperactivity during 3 minute recordings of Wistar rats (de Cabo de la Vega, Pujol et al. 1995) and in 20 minute recordings of Wistar rats (Nasello, Machado et al. 1998). The latter study also found that males had longer periods of inactivity. Female Fischer and Lewis rats tested over 3 minutes seemed to be hyperactive compared to males though this result did not reach significance and the rats in this study were extensively handled (Stöhr, Schulte Wermeling et al. 1998). Males made more entries to the inner-zone in all three temporal analyses and spent significantly longer in the inner-zone in the five and ten minute analyses. This is indicative of a sex-specific confidence in exploration away from the 'safer' edges of the arena which has been demonstrated in other studies, i.e. a reduction in anxiety-like behaviour (Beck and Luine 2002).

The findings from this phase demonstrate that isolates are in fact hypoactive in their very short term response to novelty. This provides further evidence to support motor abnormalities in SIR modelling which may have implications for schizophrenic symptomatology. It seems that isolates initially freeze and then go on to equalise in activity compared to socialised animals. It has been demonstrated that isolated animals take longer to habituate to a new environment (Powell, Swerdlow et al. 2002). Perhaps it is this failure of isolates to habituate which may underlie their relative hyperactivity in the longer term as is reported in other studies.

4.2.2 PHASE 2- OBJECT FAMILIARISATION

This phase of testing was intended not only as a chance for the animals to familiarise themselves with the two similar objects but also to demonstrate that there should be no preference between the two identical objects.

No housing differences were found in the one minute or five minute analyses of this phase. Female hyperactivity was evidenced as a greater distance travelled in both temporal analyses and additionally in the five minutes females made more entries into the quadrants containing the objects than males. All groups spent more time in the two quadrants containing the objects than in the two empty quadrants in both temporal analyses.

The female hyperactivity was consistently evident in both the first minute and five minute data analyses reinforcing the sex effect which was discussed in the context of phase 1; this was

reinforced by the increased quadrant crossings in total made by the females during the five minutes. The test to compare the time spent in the object-containing quadrants versus the empty quadrants was used as a proof of concept to determine if all animals detected these objects as salient. This proof was sustained in both temporal analyses indicating that the rats acted in a way which allows for parallels to be drawn to human studies (Clark, Geffen et al. 1987).

4.2.3 PHASE 3- NOVEL OBJECT RECOGNITION

This phase of testing addressed the primary aim of the study and provided information on the attentional responses of the isolated animals. Responses to a novel object were recorded based on more explorative centre-point detection and more investigatory nose-point detection.

A number of housing differences were evidenced in this phase of testing. In the first minute analysis isolates spent longer in the quadrant where the novel object was located whereas socialised groups did not show a quadrant preference. All groups did however make a greater number of approaches and spent longer with the novel object than with the familiar object. In the five minute analysis isolates spent longer with the novel object and the socialised animals showed no preference. In the five minute analysis all groups except isolated males made more approaches to the novel object. With regard to the distance travelled, once again, isolates were found to be hypoactive in the first minute of the phase. Females were found to be hyperactive compared to males in both temporal analyses and females also made more quadrant entries in total than males over the five minutes. The results of the tests for novelty preference are summarised in Table 8.

In the first minute isolate groups spent more time in the quadrant where the novel object was situated than in the quadrant containing the familiar object. This effect was not sustained in the five minute analysis. During the first minute all groups spent more time with the novel object itself as measured by nose-point detection. In the five minute analysis only the isolated groups spent more time with the novel object. The result implies that during the first minute the isolates responded to the novelty of the object as well as its surrounding area whereas the socialised animals are able to direct their attention to the novel object specifically. This may be relevant to schizophrenia as similarly impaired performances in attentional orienting and distractibility studies are a common finding. This is evidenced as a reduction in the task-relevant event-related potential P3 amplitude during imaging studies (Laurens, Kiehl et al. 2005). This is in line with a study which demonstrated

increased exploration of novel objects by isolates (Sahakian, Robbins et al. 1977) though this was measured as number of entries to the quadrants as opposed to the time spent. Other groups have found the opposite to be true, male and female adult S-Ds were both found to spend less time with novel objects compared to socialised animals, however the isolation period used in this study was only for 10 days prior to behavioural testing (Douglas, Varlinskaya et al. 2003).

By the time five minutes had elapsed it appeared that the socialised animals had habituated to the novel object and it was no longer salient compared to the familiar one. The isolates on the other hand continued to show preference for the novel object but were not spending an increased time in its surrounding area. Longer habituation periods are typical of isolates (Powell, Swerdlow et al. 2002) and our data supports this.

Novel object studies often do not state the specific point on which the tracking of the animal was based; this study provides simultaneous information about close-up 'investigation' as well as more general 'exploration' stimulated by novelty. This allows for an improved differentiation between these two terms which are sometimes used inconsistently between studies. Of course there are drawbacks to using an automated system for behavioural analysis as Ethovision can only detect distance of the rat (either centre-point or nose-point) from the object as an indicator of activity. However, since this was kept consistent between all recordings it was considered preferable to manual observations which would have been subject to more variable human bias. Most often in the literature the NOR test is used to provide data on memory function when the inter-trial interval is varied. Studies have shown that after isolates lose their ability to discriminate between the novel and familiar object more easily than socialised animals as the inter-trial interval is increased (McLean, Grayson et al. 2010). The present study reinforces the use of the NOR testing to provide data on attentional function by making comparisons between time periods and also using different body tracking parameters. The discrimination indices did not reach the level of significance in any of the tests (one minute, five minutes, novel object or novel quadrant) however they do show a trend for increased novelty preference by the isolates. The novelty data implies that SIR was able to induce an effect on the way in which isolates directed their attention compared to socialised animals. A difference in the response was seen in both one minute and five minute analyses with each of these time periods demonstrating a distinct effect of the housing condition.

No differences were found when the latencies to approach the objects for the first time were tested. Visually, (Figure 36) all groups seemed to approach the novel object sooner than the familiar object though this was not significant. A previous study demonstrated that isolated males took

significantly longer to contact the novel object than socialised males and no differences between the female groups were found (Douglas, Varlinskaya et al. 2003).

The findings for the distance travelled in phase 3 are consistent with the previously discussed findings from phase 1. Isolates showed decreased locomotor activity in the first minute and females were hyperactive in both the one minute and five minute analyses. Females also made more total quadrant entries than males which was consistent with the result in phase 2.

4.2.4 ALL PHASES DISTANCE TRAVELLED

Total distances travelled by each group were compared between the three phases for both the first minute and cumulative five minutes. These tests were to give an indication of how activity changed after repeated exposures to the testing arena, thus providing information on habituation (Table 9 and Table 10).

In the analysis of the distance travelled in the first minute between all phases it was found that isolates were hypoactive compared to socialised animals, the same result was not true of the five minute analysis. In both temporal analyses females were shown to be hyperactive compared to males. When the distances between the phases during the first minute were compared it was found that all animals were hypoactive in phase 1 compared to phases 2 and 3. Contrastingly, in the five minute analysis it was found that all animals were hypoactive in phase 3 compared to phases 1 and 2.

This study repeatedly demonstrated a housing effect induced by SIR which is underreported in the literature. Isolates display hypoactivity as their immediate response to a novel environment. This result demonstrates that a variety of information which can be drawn from a single measurement as simple as distance travelled. The one minute analysis provides an indicator of novelty response whereas the more commonly used 5-10 minute analyses give an idea of habituation (Brenes, Padilla et al. 2009). Studies over a course of hours on the other hand can be used to gain information about baseline activity (Gentsch, Lichtsteiner et al. 1981).

The most consistent finding when the distances travelled were analysed within and between phases was that females were hyperactive compared to males. The only time that his was not the case was during phase 1, open-field exploration, for the five and ten minute analyses.

When the total distances travelled were compared across the three phases of the test a similar trend was evident for all groups for both the one minute and five minute analyses. During the first minute of testing it was found that the distance travelled during phase 1 was significantly less than the distance travelled in both phase 2 and phase 3. This is indicative of initial freezing behaviour of all groups during the first exposure to the novel environment and increased exploratory activity in the first minute of subsequent phase 3 when the objects were introduced. Contrastingly, during the cumulative five minutes of testing it was found that the distance travelled during phase 3 was significantly less than the distance travelled in both phase 1 and phase 2. This implies that the inactivity or freezing in response to the novel environment was short-lived and the animals increased their exploration as the phase continued. The reduced distance travelled during the five minute analysis of phase 3 may be due to habituation to the arena and the objects and perhaps fatigue or boredom as phase 2 and 3 were performed on the same day within 90 minutes of each other.

4.3 *IN-VITRO* SUPERFUSION

4.3.1 SF 1- GLUTAMATE-STIMULATED [^{3}H]NE RELEASE IN HIPPOCAMPUS AND PREFRONTAL CORTEX

It was found that in both the hippocampus and prefrontal cortex that the first glutamate stimulation induced a significantly larger release of [^{3}H]NE than the second and third stimulations, supporting past papers (Howells and Russell 2008, Howells, Bindewald et al. 2009). It has been speculated that the decreased [^{3}H]NE release in response to glutamate is due to a decrease in [^{3}H]NE pool within the terminal varicosities and internalization of AMPA receptors resulting from the hyper stimulation (Howells and Russell 2008). It was also found that the first glutamate stimulation in the hippocampus caused a larger release of [^{3}H]NE than in the prefrontal cortex, similarly shown in (Howells, Bindewald et al. 2009).

No housing differences were found between the groups in the first superfusion experiment, glutamate stimulated release of [^{3}H]NE from hippocampal and prefrontal cortex tissue. No other SIR studies have measured glutamate-stimulated release of [^{3}H]NE in the hippocampus and prefrontal cortex. With the lack of finding, however with support from the literature that attentional

dysfunction is apparent in SIR and therefore there should be changes affected in the locus-coeruleus NE system termini, further experiments were developed to interrogate this.

4.3.2 SF 2- GLUTAMATE-STIMULATED [^{3}H]NE RELEASE IN HIPPOCAMPUS IN PRESENCE OF MK-801 AND/OR CNQX

To further interrogate the role of glutamate stimulated release in the SIR model, investigations to the role of glutamate ionotropic receptors in the release of [^{3}H]NE within the hippocampus were formed. Four different stimulation conditions were used; glutamate alone as a control, glutamate in combination with CNQX (an AMPA receptor antagonist), glutamate in combination with MK-801 (an NMDA receptor antagonist) and glutamate in combination with both CNQX and MK-801.

No housing differences were evident in these superfusion experiments. A sex effect was evident for the release of [^{3}H]NE resulting from control condition stimulations and when glutamate was in combination with CNQX. The release of [^{3}H]NE in females was greater than in males for these conditions. An overall increase in the [^{3}H]NE release in females was evident when the first stimulations of all four stimulation conditions were compared. These sex differences found in part agree with previous work from our laboratory, it was found that [^{3}H]NE release in the hippocampus of female S-Ds was greater than in males in response to stimulated with glutamate (Sterley, Howells et al. 2013).

As with the first superfusion experiments the release of [^{3}H]NE resulting from the first stimulation was greater than the release of [^{3}H]NE resulting from the second and third stimulations. This was the case for all of the four stimulation conditions. In the CNQX condition there was also found to be a difference between release of [^{3}H]NE resulting from the second and third stimulations. The third stimulation caused a significantly smaller release of [^{3}H]NE than the second. In work published from the same laboratory utilising the same method and concentrations it was found that CNQX caused a significant decrease in glutamate stimulated [^{3}H]NE release and that MK-801 caused a significant increase in [^{3}H]NE release (Howells and Russell 2008). When used in combination CNQX and MK-801 lead to an overall decrease in [^{3}H]NE release. From these results it was reported that glutamate-stimulated [^{3}H]NE release was AMPA but not NMDA receptor dependent. These results were however obtained from spontaneously hypertensive rats (SHR). A

strain characterisation revealed that S-Ds are less responsive overall to glutamate-stimulated [^{3}H]NE release (Howells and Russell 2008, Sterley, Howells et al. 2013). This low responsiveness may account for the lack of difference in the present study. There was also a low n for this part of the experiment which may have occluded any real differences In order to further study receptor types mediating glutamate stimulated [^{3}H]NE release *in-vitro* in S-Ds, investigation utilising more sensitive techniques, like HPLC, may be required. The fact that CNQX caused a decrease in response between stimulations 2 and 3 provides some indication of its inhibitory effects consistent with the previously discussed work in this laboratory. However it may be that a greater concentration of CNQX is required in S-Ds in order to show a significant effect compared to control glutamate only stimulations.

These experiments were designed to address a gap in the literature; it is therefore difficult to speculate on the lack of housing results. A few SIR studies which have attempted to address ionotropic glutamate receptor contribution to NE function in the hippocampus and prefrontal cortex. One study which may be of relevance used Western blotting to measure glutamate receptor subtypes in the prefrontal cortex of female S-Ds. The results showed that the GluN1 NMDA receptor subtype and GluA1 AMPA receptor subtype had been downregulated in isolated animals (Hermes, Li et al. 2011). In an SIR gene microarray study of the prefrontal cortex in male S-Ds Homer 1 was upregulated, this gene is involved in post-synaptic NMDA receptor clustering (Levine, Youngs et al. 2007).

4.3.3 SF 3- GLUTAMATE, GABA AND KCL-STIMULATED [^{3}H]NE RELEASE IN HIPPOCAMPUS AND PREFRONTAL CORTEX

Having found no difference in the role of ionotropic glutamate receptor function within the hippocampus, the next investigation served to interrogate the interaction, priming capacity, of glutamate and GABA stimulated release of [^{3}H]NE from hippocampal and prefrontal cortex tissue. It has been previously demonstrated that the interplay of these two systems can mediate NE release (Andreasen and Lambert 1991). These experiments were intended to characterise the interplay between GABA and glutamate, respectively the mammalian brains major excitatory and inhibitory

neurotransmitters . These stimulations were followed by a high concentration potassium stimulation to trigger the release of [^{3}H]NE from presynaptic reserves.

A housing difference was found when the first and second stimulation conditions were compared in the hippocampus. This effect was not sustained in post-hoc testing. In the hippocampus it was also found that the first glutamate stimulation resulted in a greater release of [^{3}H]NE than the first GABA stimulation, this difference was not found for prefrontal cortex. The first glutamate stimulation was found to cause a greater response in the hippocampus than in the prefrontal cortex. No differences were found between the brain areas in response to the first GABA stimulation. A sex difference was found in response to the KCl stimulation where the response in males was greater than in females. It was also found that [^{3}H]NE release resulting from KCl stimulations was increased in the prefrontal cortex compared to the hippocampus. A number of interactions were also found in these experiments which were not significant in post hoc testing.

When the hippocampal tissue was stimulated with glutamate and then GABA or GABA and then glutamate an overall housing difference was evident where [^{3}H]NE release by socialised animals was greater than in isolated animals. This result demonstrates that the global release of NE in the hippocampus of isolates may be reduced. The fact that this result was not obtained when stimulating with glutamate alone but after a combination of stimulations with glutamate and GABA may provide a more realistic model for *in-vivo* neurochemical function. This result is therefore a novel finding from this study as GABAergic function in relation to the NE system is understudied in the context of SIR. This result seems to be in agreement with the housing-brain area interaction evidenced when the GABA stimulation 1 data was compared, though again this was not followed through to post hoc tests. From the graphs it appears that GABA-stimulated [^{3}H]NE release less effective in the hippocampus of isolates than in socialised animals. In the past it has been demonstrated that though GABA is typically thought of as an inhibitory it is able to stimulation NE release during *in-vitro* hippocampal studies (Mc Fie, Sterley et al. 2012). This may be due to the effect of disinhibition of GABAergic signalling at interneuron level (Barik and Wonnacott 2006). This was also the case in our study where GABA was able to evoke [^{3}H]NE release in both brain areas. GABA mediated NE release in the hippocampus is thought to be exclusively via $GABA_A$ receptors (Raiteri, Raiteri et al. 2002). This study labelled the hippocampus of male S-Ds with [^{3}H]NE and found that release was GABA concentration dependent. They posited that a GABA heterocarrier enabled this NE release. Another study used *in-vivo* microdialysis to ascertain the effects of ionotropic glutamate receptor agonists and antagonists on extracellular NE levels in the hippocampus in the presence of a $GABA_A$ antagonist- bicuculline. It was found that bicuculline

was able to effectively antagonise the ability of NMDA to cause a decrease in basal extracellular NE levels. The suggested reason for this was that NMDA receptors are located on GABAergic interneurons which serve to mediate glutamate-stimulated NE release in the hippocampus through reciprocal activation (Dazzi, Matzeu et al. 2011). It may be that the dysregulation of this system caused differential results of GABA-mediated of [^{3}H]NE release in the isolates in of this study. This effect warrants further study to investigate the role of GABA-NE. GABA antagonists have been shown to lead to an overall decrease of nicotine-stimulated NE release in another microdialysis study in the hippocampus, further strengthening the importance of GABA in NE transmission (Fallon, Shearman et al. 2007). One other study further strengthened a role for GABA in NE activity in the hippocampus by demonstrating that in hippocampal slices, NE was able to stimulate GABAergic neurons causing an increase in the generation of inhibitory post synaptic potentials (Andreasen and Lambert 1991). It would be of particular interest to investigate how bicuculline might differentially affect [^{3}H]NE release in a future superfusion study utilising glutamate and GABA-stimulation to see how $GABA_A$ transmission in the hippocampus might be altered by SIR.

In the hippocampus it was found that the [^{3}H]NE release resulting from the first stimulation with glutamate was greater than the [^{3}H]NE release resulting from the first stimulation with GABA for all groups, though there were no differences in stimulating effect of GABA and glutamate in the prefrontal cortex. This lack of difference in the prefrontal cortex may have been due to the lower total [^{3}H]NE release that was demonstrable in this region. In both the first and the third superfusion experiments the total [^{3}H]NE release was found to be greater in the hippocampus than in the prefrontal cortex. The greater [^{3}H]NE release activity in the hippocampus may have made the difference between glutamate and GABA-stimulated activity more pronounced, contributing to the significant effect.

In the test between KCl stimulations in the brain areas a sex difference was found such that KCl-stimulated [^{3}H]NE release in males was greater than in females. A difference between the brain areas was also evident. KCl-stimulated [^{3}H]NE release was greater in the prefrontal cortex, this was in contrast to the finding that glutamate-stimulated [^{3}H]NE release was greater in the hippocampus. This disparity is likely to be facilitated by different receptor populations in the two areas. KCl-stimulated [^{3}H]NE release may be mediated by negative feedback inhibition of α_2-adrenoceptors in NE neurons which act as autoreceptors (Russell, Allie et al. 2000). This was characterised using a similar superfusion technique where KCl-stimulated [^{3}H]NE release took place in the presence of an α_2-adrenoceptor agonist UK 14,304 which led to a reduction in KCl-stimulated [^{3}H]NE release.

In the context of the present study a reduced population of α_2-adrenoceptors in the prefrontal cortex may be responsible for the increased KCl-stimulated [^{3}H]NE release compared to the hippocampus. In a similar study in the SIR model, the effect of a high K^+ stimulation on NE was investigated in isolated male Lister Hooded rats to characterise noradrenergic function and the Ca^{2+}-dependency of the response (Fulford and Marsden 1997). The rats were isolated from p21 for 4-6 weeks and then decapitated and the hippocampi were submerged in Krebs buffer. In contrast to the present study, after slicing, the tissues were centrifuged to form pellets. The pellets were incubated with solutions containing either 30 mM K^+, 10 µM clonidine (an α_2-adrenoceptor agonist) or 10 µM idazoxan (an α_2-adrenoceptor antagonist) for 20 minutes and then centrifuged again. Finally the supernatants underwent HPLC to determine NE concentrations. No differences were found with regard to the basal or high K^+ stimulated release of NE in the hippocampus in socialised and isolated rats. Basal as well as K^+ stimulated release of NE in the hippocampus was shown to be Ca^{2+}-dependent. The α_2-adrenoceptor agonist had no differential effects on NE release whereas the α_2-adrenoceptor antagonist caused a significantly greater increase in NE release in isolates compared to basal release than it did in socialised animals. It was postulated that this effect is mediated by increased that presynaptic α_2-autoreceptor sensitivity in the hippocampus due to isolation. In the present study the differences found in NE release in the presence of GABA and glutamate demonstrates the interaction of these systems and their aberration following isolation.

Overall these superfusions were the most revealing of NE function in the context of SIR as they revealed a tentative difference such that overall presynaptic NE activity was reduced in the hippocampus of isolates in response to sequential stimulations with glutamate and then GABA or GABA and then glutamate. This may be mediated by attenuated $GABA_A$ receptor expression in the dentate gyrus but increased glutamate NMDA and kainate receptor expression in the CA1 and CA3 regions of the hippocampus as demonstrated in a previous study (Iwata and Yamamuro 2016) in combination with increased α_2-autoreceptor sensitivity.

4.4 ELISA AND BCA PROTEIN ASSAY

The overall the concentrations of glutamate and NE in the hippocampus and prefrontal cortex tissue where investigated to determine whether absolute concentrations of NE were affected by SIR

No housing differences were found in relation to neurotransmitter concentrations in the hippocampus and prefrontal cortex. HPLC studies have found differences between socialised and isolated animals levels of NE in the ventral striatum (Brenes, Rodríguez et al. 2008) but not in the nucleus accumbens, the prefrontal cortex or the hippocampus (Brenes, Padilla et al. 2009, Kirkpatrick, Marshall et al. 2014). Another study utilising single isotope radioenzymatic assay found there to be a reduction in NE in the hypothalamus of isolated animals but not in the hippocampus (Dronjak and Gavrilovic 2006).

The only differences found were NE concentration differences between the sexes. In the hippocampus the NE concentration in the wet weight of tissue was greater in females than in males, a similar effect was found for the NE concentration in the protein of the hippocampus where values for the female socialised group were greater than the male socialised group. The concentration of NE in the wet weight of tissue in the prefrontal cortex was greater in males than in females. With regard to the sex differences evidenced in the NE concentrations in the hippocampus and prefrontal cortex, it is surprisingly difficult to find any evidence (supportive or otherwise) in age and strain-matched tests in both sexes. In one previous study of NE concentrations using HPLC in the hippocampus and prefrontal cortex of Wistar rats, sex differences were not found (Del Pino, Martínez et al. 2011). However these data support a differential expression of NE for different brain areas by sex, further study is required to understand the role of the differential concentrations in NE found.

There were no differences between the groups for any of the glutamate concentrations measured. Given its abundance and importance throughout the nervous system it is not surprising that isolation had no effects on the total concentration of glutamate in the hippocampus and prefrontal cortex. A similar result was found in another study where a microdialysis probe was placed in the prefrontal cortex to collect extracellular fluid in order to quantify glutamate concentrations (Melendez, Gregory et al. 2004). It is more likely that alterations in glutamate function happen due to receptor plasticity and functionality as evidenced in glutamate receptor studies. Western blots of glutamate receptor types in male S-D hippocampus tissue have revealed reduced expression of GluN1, GluA1 and GluA2 in isolates, these changes are proposed to attenuate synaptic plasticity processes (Wang, Huang et al. 2017). This decrease in AMPA receptor subtypes in the hippocampus of male isolated rats has been replicated for GluA1 and GluA2 (Sestito, Trindade et al. 2011). However in a study to quantify the mRNA expression of NMDA receptor subunit NMDAR1A in isolates in the hippocampus, prefrontal cortex and striatum, no differences were found (Hall, Ghaed et al. 2002). In another paper, RNA from the hippocampus and prefrontal

cortex of isolated male S-Ds was extracted and analysed with real-time polymerase chain reaction. It was reported that in the hippocampus NMDA subtypes GluN2A and GluN2B were increased in isolates and in the prefrontal cortex GluN2A subtypes were decreased (Zhao, Sun et al. 2009). A decrease in GluA1 has also been demonstrated in the medial prefrontal cortex of isolated male rats (Sarkar and Kabbaj 2016). A similar result was found in the prefrontal cortex of isolated female S-Ds where GluA1 and GluN1 populations were reduced (Hermes, Li et al. 2011). Glutamate receptor subunit studies have also been carried out in the prefrontal cortex indicating a reduction of the subunits mGluR1, mGluR5 and mGluR2/3 in male isolates and an increase in mGluR1 (Melendez, Gregory et al. 2004). Further studies are required to further characterise glutamate and NE receptor populations in brain areas related to attention in the SIR model.

4.5 LINKING BEHAVIOURAL AND NEUROCHEMICAL FINDINGS

So far the scope of the discussion has stayed within the context of single findings within each experiment. This section will draw together related findings from the experiments discussed above which were designed to probe the attentional system in the SIR model.

It was found that there was an overall decrease in glutamate and GABA-mediated release of NE in the hippocampus of isolates during superfusion. Isolates were also found to demonstrate abnormal attentional behaviours, evidenced by the attribution of salience to a novel object as well as its surrounding area and slower habitation to novelty compared to socialised animals. It has been previously demonstrated that phasic release of NE in LC varicosities is required for optimal attentional processing (Howells, Stein et al. 2012). It has been shown that the stimulation of glutamatergic neurons by GABA interneuron activity can alter NE release in the hippocampus (Dazzi, Matzeu et al. 2011), it has also been shown that adrenoceptors play a role in regulating GABA activity in the hippocampus (Andreasen and Lambert 1991). In the superfusions with glutamate alone, no effects of the isolation protocol were demonstrated. This implies that abnormal negative feedback regulation of glutamatergic transmission by GABA might contribute to an overall decrease in NE function in hippocampal circuits which may underlie attentional dysfunction seen in isolates.

In the present study the hyperactivity of females in the behavioural tests may be explainable by the increased NE activity in the hippocampus. It has been demonstrated in a previous studies that hyperactivity can be experimentally induced by increasing NE in the hippocampus (Flicker and Geyer 1982, Suwabe, Kubota et al. 2000). In the neurochemical experiments a few sex differences were apparent. In the superfusion experiments the release of [^{3}H]NE was greater in the hippocampus of females after; stimulations with glutamate alone, stimulations with glutamate in the presence of CNQX and overall when the results from the first stimulation of the four different stimulation conditions of the superfusion 2 experiments utilising different glutamatergic antagonists were combined. Contrastingly, the release of [^{3}H]NE was greater in both the hippocampus and prefrontal cortex of males when the stimulation medium was KCl. Differences in the response of the brain areas was also evident in the superfusion experiments. Release of [^{3}H]NE in response to the first glutamate stimulation was found to be greater in the hippocampus than in the prefrontal cortex. This was the case in the first and the third superfusion experiments. Further, release of [^{3}H]NE in response to stimulation with KCl was found to be greater in the prefrontal cortex than in the hippocampus. Then the concentration of NE in the hippocampus was found to be increased in females while the concentration of NE in the prefrontal cortex was found to be increased in males.

Taken together these data may have implications for differential NE function in the hippocampus and prefrontal cortex of the sexes. It seems that in females there is an elevated NE concentration in the hippocampus as evidenced by the ELISA data and this is accompanied by greater release of NE in response to glutamatergic stimulation as evidenced by the superfusion data. Additionally it was demonstrated in the superfusion experiments that overall glutamate-stimulated release of NE is greater in the hippocampus than in the prefrontal cortex. This increased NE activity in the hippocampus of females has been shown to be dependent on female sex hormones. In a study of ovariectomised female rats decreased norepinephrine levels in the hippocampus were rescuable upon housing with estradiol (Bowman, Ferguson et al. 2002). In males an elevated concentration of NE was found in the prefrontal cortex in the ELISA study and in the superfusions males had an elevated response of [^{3}H]NE release when stimulated with KCl . KCl was found to have the greater effect on [^{3}H]NE in the prefrontal cortex compared to the hippocampus. This is fitting with the literature as it was found that K^+ -stimulated NE release in the olfactory bulb was decreased in castrated rats (Guan and Dluzen 1991), interestingly this effect was not rescuable after the administration of testosterone.

To conclude, with regard to sex differences found, females showed increased NE responsivity and concentration in the hippocampus whereas in males showed increased NE responsivity and

concentration in the prefrontal cortex. In the context of existing literature this differences are posited to be related to sex hormones and additionally it has been suggested that the increased NE activity in the female hippocampus may contribute to the finding of female hyperactivity in the behavioural experiments of this study. Future studies would likely benefit from including the measurement of sex hormones as these may provide the necessary insights to understand the hyperactivity seen in females, the differential release of [^{3}H]NE, and NE concentration differences. Additionally future studies are suggested which probe the relationship between glutamate, GABA and norepinephrine activity using agonists, antagonists and receptor population studies in brain areas related to attention to further understand these dysfunctional system in isolated animals.

4.6 ULTRASONIC VOCALISATIONS

These experiments were intended as secondary aim of the study to assess calling behaviour in response to a novel environment to provide an insight into one of the negative symptoms of schizophrenia; social withdrawal.

No sex or housing differences were demonstrable between the groups during ultrasonic vocalisation tests at 22 kHz. Technical issues with the recording equipment, a low sample number and the introduction of a correction factor because of apparent differences in the sensitivities of the detectors may have occluded group differences. Another confounding factor in the USV test was the presence of two animals (one socialised and one isolated) which were tested at the same time. The purpose of this was to stimulate communication, though it is difficult to say how well the animals could hear and or see each other since they were in two separate cylinders. This poses the question of whether they were communicating to each other or rather simply vocalising in response to being in a new environment and whether the results should be interpreted as a novelty response or social function. Numerous studies have been performed investigating how rats respond to the affect of other rats. Responses to ‘negative’ affect vocalisation at 22 kHz and ‘positive’ affect 50 kHz have been investigated as part of empathy studies and the results showed that the affect of the listening rat was altered after listening to recordings (Saito, Yuki et al. 2016). Therefore, if in the present study the rats could indeed hear each other they may have influenced each other’s behaviour leading to no differences being found.

USV recordings from SIR model animals is not yet commonplace in the literature and variety of testing conditions have been used. Results from existing 22 kHz studies in SIR have shown that the total number of calls was reduced in isolates when they were placed in a novel environment (Nunes Mamede Rosa, Nobre et al. 2005). Another study also found that isolates made fewer calls of 22 kHz in response to a novel environment and also that the duration of the calls was significantly reduced compared to socialised controls (Tomazini, Reimer et al. 2006) .

From an examination of the existing literature it seems that trying to infer human-like emotional states from USV data in rats is somewhat problematic. Where SIR has been found to cause differences in USV this should be taken at face value as an 'abnormal' response. For this reason, in future studies, in order to extrapolate relevant information for human conditions it may be prudent to couple USV testing with other behavioural test (e.g. NOR). By combining audio and visual data this may help to disentangle how rats vocalise when not in the presence of other rats. This could provide more robust integrated behavioural information, for example: whether an increase in the number of calls is correlated to hyperactivity or freezing behaviour.

CHAPTER 5
-CONCLUSION-

In the present study the SIR model was found to induce a number of changes in behaviour and neurochemistry. The primary aim of the study was to investigate whether the attentional deficits associated with the cognitive symptoms of schizophrenia were evident in isolated animals by investigating behaviour related to the function of the locus-coeruleus norepinephrine system. In order to assess attention a novel object recognition behavioural test was used. Often this test is used to study memory but in the present study it was adapted to provide information on attentional processing by measuring close investigatory behaviour and more general exploratory behaviours utilising two different types of rat body movement tracking. Additionally, parameters of this test were analysed at multiple time points to provide information about how the reactions of the rats to novelty changed with time. In the first minute of the test it was found that isolated animals attributed salience to the area surrounding a novel object as well as to the object itself. Socialised animals on the other hand directed their attention specifically to the novel object. In the cumulative five minute analysis of the novel object test isolated animals continued to direct their attention to the novel object whereas socialised animals no longer demonstrated this preference indicating different habituations to novelty. These results imply that the attentional response to novel stimuli is abnormal in isolated animals. Additionally, an overall effect was found in behavioural testing where isolates were hypoactive compared to socialised animals in the first minute after they were placed into an environment outside the home cage. No differences were found between the groups when longer term analyses of locomotor activity were conducted. This very short-term novelty-induced freezing response by isolates has not yet been reported in the literature and implies an abnormal response to environmental novelty which changes with time.

The neurochemical aspects of this study were designed to address whether the systems which are involved in attentional processing had been altered by isolation rearing. These experiments were the first of their kind and provided tentative information to suggest that isolated animals has decreased NE activity in the hippocampus. This was evidenced in a superfusion study where stimulation hippocampal tissue by sequential stimulations with glutamate and GABA were found to cause an overall reduction in the amount of [^{3}H]NE released by isolates. In this study the overall concentration of NE in the hippocampus measured by ELISA was found not to differ between the

housing groups. This result warrants further investigation of receptor populations in the hippocampus which may be responsible for this reduction in NE activity if it is not simply related to a reduction in the total NE concentration. A future study could utilise combinations of glutamatergic and GABA receptor agonists to try and ameliorate the reduction of NE activity in the hippocampus of isolates. Additional studies with bicuculline- a $GABA_A$ antagonist will help to identify which type of receptors are involved in aberrant GABAergic processing in isolates.

It is postulated that dysregulation of negative feedback circuits involving GABAergic interneurons synapsing on glutamatergic neurons the in the hippocampus may be responsible for attentional deficits evidenced in the isolates of this study. This finding may provide a target for the study (and potentially treatment) of the cognitive symptoms of schizophrenia.

Other interesting results of the study pertained to sex differences. It was found that females had increased NE functionality and concentration in the hippocampus whereas in males had increased NE functionality and concentration in the prefrontal cortex. In the context of existing literature this differences are posited to be related to sex hormones and additionally it has been suggested that the increased NE activity in the female hippocampus may contribute to the finding of female hyperactivity in the behavioural experiments of this study.

Overall the present study was able to successfully address its primary aim to assess the behavioural and neurochemical aspects of the attentional system in the SIR model. This was achieved by the demonstration that both male and female isolated animals showed abnormal attentional responses to novel objects in a behavioural paradigm as well as an overall reduction in NE release in response to glutamate and GABA stimulations in the hippocampus. Future studies will target GABA regulation of hippocampal NE release in order to better understand dysregulated attention in the socially isolated rat model of schizophrenia. This may help to provide a better mechanistic understanding of the cognitive symptoms in the human disorder of schizophrenia.

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-APPENDICES-

UNIVERSITY OF CAPE TOWN

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http://www.health.uct.ac.za/fhs/research/animalethics/

03 February 2015

Dr FM Howells
Psychiatry & Mental Health
Office 105
J-2 Block
GSH

Dear Dr Howells

PROTOCOL TITLE: LOCUS-COERULEUS NOREPINEPHRINE SYSTEM FUNCTION IN A DEVELOPMENTAL ANIMAL MODEL OF SCHIZOPHRENIA: THE SOCIALLY ISOLATED RAT

FHS AEC REF NO: 014/047

Thank you for submitting your protocol to the Faculty of Health Sciences (FHS) Animal Ethics Committee (AEC) for review.

I am pleased to inform you that the FHS AEC has **authorised** your protocol, which will terminate on **03 February 2018**

Number of animals & species: 140 Rats

Please quote the FHS AEC REF NO (above) in all future correspondence.

Please note that the authorisation of this protocol imposes the following obligations on the (PI) principal investigator:

1. To submit an annual mandatory progress report. The first annual report for this protocol is due on **03 February 2016**. The forms can be accessed from http://www.health.uct.ac.za/fhs/research/animalethics/forms
2. To submit a final mandatory report on the **03 February 2016**, please access the final report form from: http://www.health.uct.ac.za/fhs/research/animalethics/forms
3. Ensuring that all study participants perform within the confines of the procedures and experimental design of the protocol as authorised, or as amended.
4. Ensuring that all study participants comply with all applicable national legislation, UCT policies, FHS AEC policies and standard operating procedures (SOPs) and national standards (SANS 10386: 2008).
5. Ensuring that you as the PI (principal investigator) immediately alert the FHS AEC to any event involving the welfare of the animals which has occurred during the course of the study, as well as the actions that were taken to respond to these events.
6. Ensuring that you as the PI (principal investigator) alert the FHS AEC to any new or unexpected ethical issues that arose during the course of the study, and how these issues were addressed.
7. Ensuring that all study participants are registered with or have been authorised by the South African Veterinary Council (SAVC) to perform the procedures on animals, or will be performing the procedures under the direct and continuous supervision of SAVC-registered veterinary professionals or SAVC-registered para-veterinary professionals.
8. If the principal investigator or any study participant is in any way uncertain how to respond to any of these obligations or deal with any of the issues referred to above, they must consult with FHS AEC.
9. All animals found dead must be reported to the RAF on the appropriate form: http://www.health.uct.ac.za/fhs/research/animalethics/forms
10. All animals found in distress must be reported to the RAF on the appropriate form.

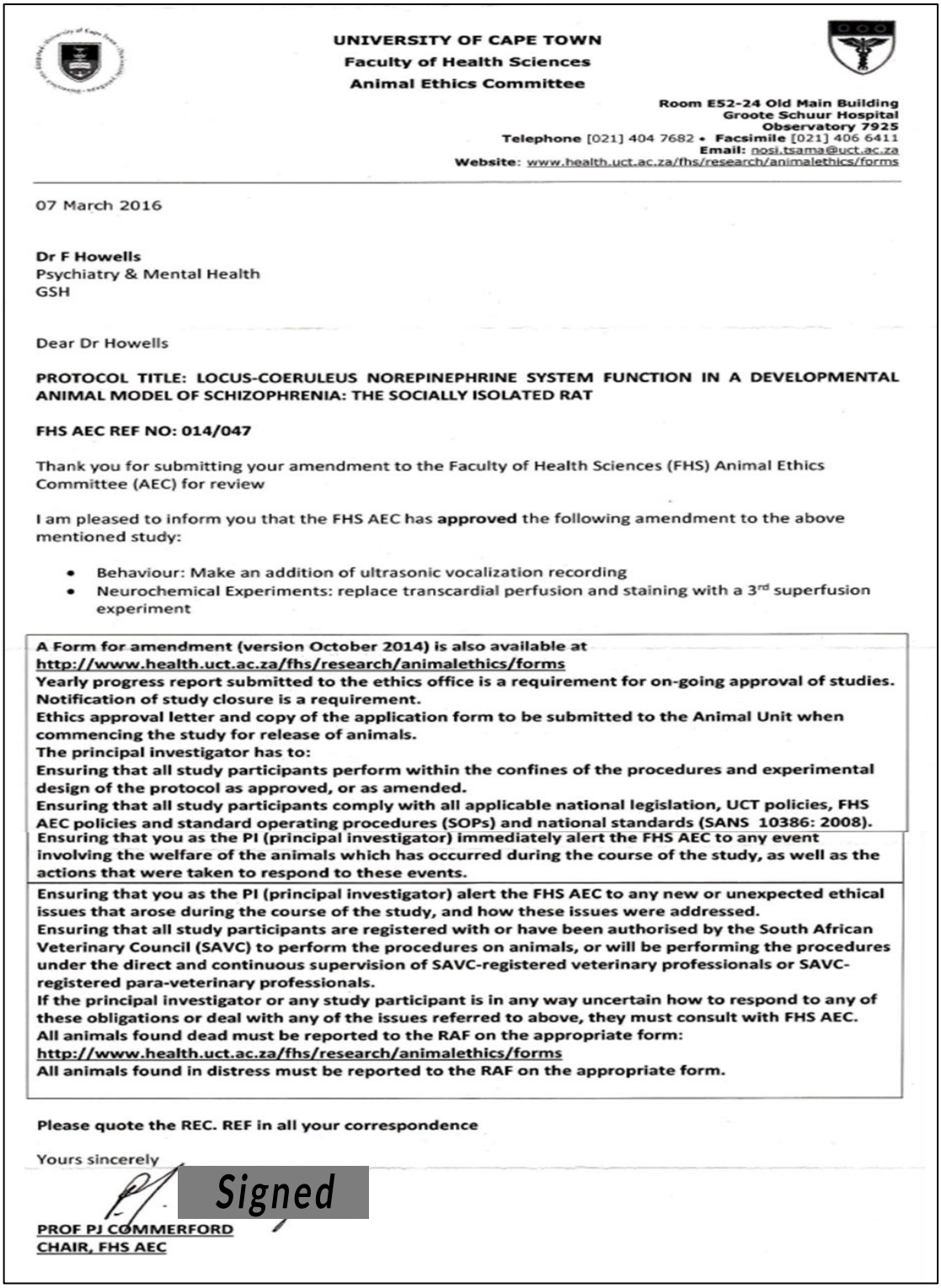
My best wishes for a successful research and /or teaching endeavour.

Yours sincerely

Signed

PROF PJ COMMERFORD
CHAIR, FHS AEC

Figure 69- AEC documentation 1/2



UNIVERSITY OF CAPE TOWN
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07 March 2016

Dr F Howells
Psychiatry & Mental Health
GSH

Dear Dr Howells

PROTOCOL TITLE: LOCUS-COERULEUS NOREPINEPHRINE SYSTEM FUNCTION IN A DEVELOPMENTAL ANIMAL MODEL OF SCHIZOPHRENIA: THE SOCIALLY ISOLATED RAT

FHS AEC REF NO: 014/047

Thank you for submitting your amendment to the Faculty of Health Sciences (FHS) Animal Ethics Committee (AEC) for review

I am pleased to inform you that the FHS AEC has **approved** the following amendment to the above mentioned study:

- Behaviour: Make an addition of ultrasonic vocalization recording
- Neurochemical Experiments: replace transcardial perfusion and staining with a 3rd superfusion experiment

A Form for amendment (version October 2014) is also available at http://www.health.uct.ac.za/fhs/research/animalethics/forms
Yearly progress report submitted to the ethics office is a requirement for on-going approval of studies.
Notification of study closure is a requirement.
Ethics approval letter and copy of the application form to be submitted to the Animal Unit when commencing the study for release of animals.
The principal investigator has to:
Ensuring that all study participants perform within the confines of the procedures and experimental design of the protocol as approved, or as amended.
Ensuring that all study participants comply with all applicable national legislation, UCT policies, FHS AEC policies and standard operating procedures (SOPs) and national standards (SANS 10386: 2008).
Ensuring that you as the PI (principal investigator) immediately alert the FHS AEC to any event involving the welfare of the animals which has occurred during the course of the study, as well as the actions that were taken to respond to these events.
Ensuring that you as the PI (principal investigator) alert the FHS AEC to any new or unexpected ethical issues that arose during the course of the study, and how these issues were addressed.
Ensuring that all study participants are registered with or have been authorised by the South African Veterinary Council (SAVC) to perform the procedures on animals, or will be performing the procedures under the direct and continuous supervision of SAVC-registered veterinary professionals or SAVC-registered para-veterinary professionals.
If the principal investigator or any study participant is in any way uncertain how to respond to any of these obligations or deal with any of the issues referred to above, they must consult with FHS AEC.
All animals found dead must be reported to the RAF on the appropriate form:
http://www.health.uct.ac.za/fhs/research/animalethics/forms
All animals found in distress must be reported to the RAF on the appropriate form.

Please quote the REC. REF in all your correspondence

Yours sincerely

Signed

PROF PJ COMMERFORD
CHAIR, FHS AEC

Figure 70- AEC documentation 2/2

140 animals were obtained from project number 014/047 for this study. An additional 36 animals from project number 014/048 were also added as the student initially designated them was unable to complete their research. This accounts for the unequal ratio of males to females overall

A.1 BODY WEIGHT

Table 11- Body weight, Data 1/2

Rat #	Sex	Treatment	Sex_Treatment	p21-28	p28-35	p35-42	p42-49	p49-56	p56-63	p63-70	p70-77	p77-84	p84-91
1	F	S	FS	40	55	87	128	157	167	187	192	205	209
2	F	S	FS	52	70	111	150	177	180	225	261	279	279
3	F	S	FS	40	60	91	132	165	172	200	221	222	241
4	F	S	FS	46	63	94	131	165	165	201	223	230	240
5	F	I	FI	49	64	104	152	162	181	210	234	264	263
6	F	I	FI	51	74	119	156	168	183	222	232	247	258
7	F	I	FI	49	70	116	157	179	184	215	239	261	257
8	F	I	FI	52	82	122	160	175	192	212	238	249	259
9	M	S	MS	37	52	87	131	128	218	277	301	317	340
10	M	S	MS	39	52	93	141	190	233	291	216	340	363
11	M	S	MS	39	57	99	146	202	225	309	333	355	374
12	M	S	MS	39	60	103	151	203	237	300	334	355	378
13	M	I	MI	61	105	169	234	275	331	357	384	402	413
14	M	I	MI	64	108	161	218	264	302	325	354	366	395
15	M	I	MI	60	102	166	228	274	321	349	372	394	422
16	M	I	MI	61	107	160	212	242	305	326	355	371	398
17	F	S	FS	39	53	85	127	142	174	203	221	224	219
18	F	S	FS	36	47	78	120	144	158	182	188	201	203
19	F	S	FS	34	44	76	113	139	179	207	224	237	243
20	F	S	FS	36	49	83	125	137	167	199	209	208	233
21	F	I	FI	34	47	85	128	144	171	192	197	219	228
22	F	I	FI	36	47	82	120	140	161	183	182	205	217
23	F	I	FI	36	49	86	125	139	171	200	222	226	228
24	F	I	FI	35	46	82	122	144	168	190	200	208	211
25	M	S	MS	32	49	84	130	174	222	241	266	288	297
26	M	S	MS	34	53	89	136	180	229	248	277	303	320
27	M	S	MS	37	51	83	127	171	216	240	271	289	304
28	M	S	MS	31	47	91	142	194	248	268	299	321	335
29	M	I	MI	29	44	77	118	161	208	230	253	268	282
30	M	I	MI	23	34	64	112	147	214	245	272	289	303
31	M	I	MI	27	38	85	132	197	236	255	285	314	331
32	M	I	MI	32	45	60	109	174	214	243	283	300	322
33	F	S	FS	38	50	82	129	150	169	189	193	210	214
34	F	S	FS	38	51	87	138	166	173	196	200	226	249
35	F	S	FS	40	49	86	131	172	173	197	207	214	225
36	F	S	FS	36	52	91	133	164	169	197	213	222	230
37	F	I	FI	34	51	85	118	157	173	194	217	221	236
38	F	I	FI	45	58	97	141	173	193	215	236	255	258
39	F	I	FI	39	54	90	133	167	178	200	230	247	249
40	F	I	FI	40	56	88	132	160	173	192	204	208	227
41	M	S	MS	36	57	107	152	185	228	256	278	298	315
42	M	S	MS	35	66	112	154	190	226	257	277	295	307
43	M	S	MS	36	65	118	163	205	240	261	277	289	297
44	M	S	MS	29	70	127	175	221	270	301	330	351	359
45	M	I	MI	34	67	103	171	210	252	285	305	331	345
46	M	I	MI	42	86	150	209	260	293	348	365	390	402
47	M	I	MI	32	62	102	154	196	240	277	301	320	340
48	M	I	MI	30	59	99	142	159	215	240	257	280	298
49	F	S	FS	43	67	103	141	167	188	200	207	212	247
50	F	S	FS	37	66	105	138	163	169	183	203	209	230
51	F	S	FS	40	66	92	124	148	182	190	207	214	221
52	F	S	FS	42	67	103	134	162	184	202	204	226	239
53	F	I	FI	40	62	100	128	152	175	185	204	210	229
54	F	I	FI	36	57	105	130	160	182	188	214	222	224
56	F	I	FI	37	55	91	129	149	162	190	197	219	222
57	M	S	MS	40	54	85	140	177	232	273	302	324	343
58	M	S	MS	46	60	79	127	158	209	250	291	294	317
59	M	S	MS	42	51	70	121	157	211	251	295	301	318
60	M	S	MS	40	53	75	119	162	212	253	291	297	322
61	M	I	MI	34	50	82	125	162	196	237	256	285	300
62	M	I	MI	36	54	94	145	195	235	276	306	326	352
63	M	I	MI	38	53	80	125	168	210	250	297	302	325
64	M	I	MI	35	45	77	125	173	215	256	297	310	333
65	F	S	FS	35	57	94	129	154	178	202	206	216	226
66	F	S	FS	44	70	110	146	149	198	210	222	235	236
67	F	S	FS	40	67	104	140	155	187	192	210	221	222
68	F	S	FS	39	65	98	133	158	195	202	210	219	236
69	F	I	FI	44	75	101	139	160	190	214	227	236	238
70	F	I	FI	45	80	124	160	198	218	228	256	262	264
71	F	I	FI	40	81	122	154	175	195	217	220	227	232
72	F	I	FI	43	72	106	141	160	187	203	215	223	228
73	M	S	MS	45	63	120	158	211	263	280	310	334	354
74	M	S	MS	47	74	116	158	198	234	253	272	291	312
75	M	S	MS	43	63	106	140	189	232	245	269	283	302
76	M	S	MS	42	65	92	129	175	209	228	258	274	298
77	M	I	MI	45	74	126	185	223	274	308	324	341	370
78	M	I	MI	46	69	115	173	212	255	293	321	346	371
79	M	I	MI	46	65	120	176	219	259	297	314	367	375
80	M	I	MI	48	78	131	184	220	270	301	329	354	388
81	F	S	FS	49	81	112	133	153	176	186	197	203	211
82	F	S	FS	47	82	110	130	153	174	182	204	210	213
83	F	S	FS	43	80	108	131	151	170	180	203	210	211
84	F	S	FS	46	80	111	130	152	172	192	206	216	220
85	F	I	FI	46	93	128	152	165	173	198	203	208	213
86	F	I	FI	46	92	123	148	163	179	199	205	217	220
87	F	I	FI	42	83	125	151	171	188	194	200	206	208
88	F	I	FI	47	84	119	127	154	166	185	190	191	232

Table 12- Body weight, Data 2/2

Rat #	Sex	Treatment	Sex_Treatment	p21-28	p28-35	p35-42	p42-49	p49-56	p56-63	p63-70	p70-77	p77-84	p84-91
89	M	S	MS	59	84	120	149	190	222	246	266	285	293
90	M	S	MS	63	87	120	139	194	234	255	276	309	321
91	M	S	MS	41	66	106	126	168	210	230	255	270	280
92	M	I	MI	53	80	128	165	201	237	260	272	300	314
93	M	I	MI	55	86	133	168	214	247	280	301	323	331
94	M	I	MI	64	97	150	193	237	272	299	324	341	358
95	M	S	MS	43	74	114	151	205	233	274	284	302	311
96	M	S	MS	41	88	127	171	217	254	296	300	307	337
97	M	I	MI	44	88	135	180	232	265	302	329	337	346
98	M	I	MI	43	87	139	184	238	275	322	351	369	383
99	F	S	FS	59	75	113	129	163	172	187	195	210	214
100	F	I	FI	50	87	116	141	162	180	192	198	210	213
73.1	F	S	FS	47	81	123	141	172	197	228	236	250	260
74.1	F	S	FS	44	65	96	113	135	157	190	194	197	205
75.1	F	S	FS	44	72	114	136	145	197	200	203	217	226
76.1	F	S	FS	49	68	106	124	143	166	194	207	212	215
77.1	F	I	FI	47	74	115	132	158	175	206	207	223	227
78.1	F	I	FI	52	80	116	151	193	224	229	240	244	248
79.1	F	I	FI	42	89	120	141	163	177	202	209	224	229
80.1	F	I	FI	46	80	122	149	168	171	217	221	230	244
81.1	M	S	MS	55	74	117	121	234	240	273	297	302	312
82.1	M	S	MS	44	77	103	131	214	225	258	283	303	311
83.1	M	S	MS	46	85	116	128	226	235	272	288	297	309
84.1	M	S	MS	50	89	120	139	243	258	302	312	320	327
85.1	M	I	MI	54	108	166	195	297	307	340	365	388	401
86.1	M	I	MI	48	87	141	193	270	285	306	333	349	350
87.1	M	I	MI	43	91	145	189	271	277	313	354	367	381
88.1	M	I	MI	48	96	145	194	269	275	311	335	354	369
89.1	F	S	FS	65	84	103	150	162	180	196	210	212	225
90.1	F	S	FS	59	86	97	127	134	163	188	197	201	215
91.1	F	S	FS	59	77	116	143	146	166	176	195	211	217
92.1	F	I	FI	61	73	119	161	163	176	186	206	197	209
93.1	F	I	FI	66	80	114	160	164	186	202	209	219	225
94.1	F	I	FI	57	76	108	148	149	169	186	200	210	222
95.1	M	S	MS	43	64	167	177	220	268	304	310	316	337
96.1	M	I	MI	43	67	172	186	235	274	313	327	345	362
97.1	F	S	FS	53	97	133	163	174	190	207	219	232	235
98.1	F	S	FS	54	85	113	149	179	183	194	200	208	217
99.1	F	I	FI	56	98	115	160	195	203	215	227	231	240
100.1	F	I	FI	45	78	126	139	154	167	176	188	198	200
101	M	S	MS	38	54	62	87	104	137	179	238	256	289
102	M	S	MS	36	56	79	104	126	167	224	274	298	310
103	M	S	MS	34	60	79	105	130	170	217	253	269	300
104	M	S	MS	38	59	77	111	128	173	226	270	302	315
105	M	S	MS	43	58	79	109	134	182	233	284	311	332
106	M	I	MI	39	63	93	127	156	209	256	294	313	325
107	M	I	MI	30	52	83	122	145	202	263	298	326	341
108	M	I	MI	33	54	79	107	134	190	247	269	285	302
109	M	I	MI	36	63	91	127	161	222	278	330	343	360
110	M	I	MI	36	57	83	124	147	202	255	299	308	323
111	F	S	FS	34	42	56	74	107	140	166	192	198	206
112	F	S	FS	36	47	61	82	93	126	155	189	197	206
113	F	S	FS	36	55	83	112	131	160	176	195	216	238
114	F	S	FS	38	56	85	109	133	151	178	206	210	214
115	F	S	FS	30	49	72	97	117	151	177	198	208	217
116	F	I	FI	34	52	80	115	133	157	168	185	194	205
117	F	I	FI	36	50	75	110	133	164	187	212	226	234
118	F	I	FI	34	60	74	106	126	160	184	205	214	223
119	F	I	FI	34	52	70	104	123	155	176	192	198	208
120	F	I	FI	37	53	73	101	122	157	182	209	216	227
121	F	S	FS	41	57	74	95	139	168	213	222	246	253
122	F	S	FS	57	71	96	115	165	180	191	204	219	234
123	F	S	FS	49	77	99	117	161	187	205	223	242	259
124	F	S	FS	51	74	95	116	153	178	203	216	224	237
125	F	I	FI	44	68	96	126	162	184	213	220	232	242
126	F	I	FI	45	70	101	135	171	196	219	231	241	246
127	F	I	FI	40	63	96	112	144	177	196	199	202	223
128	F	I	FI	53	77	119	142	176	205	224	233	240	263
129	F	S	FS	38	52	81	116	162	170	182	206	222	222
130	F	S	FS	28	38	64	106	139	144	167	194	207	207
131	F	S	FS	28	38	60	106	137	148	169	187	213	213
132	F	S	FS	38	50	78	121	155	169	181	202	214	226
133	F	I	FI	47	63	103	145	170	174	179	211	224	227
134	F	I	FI	28	40	72	106	137	141	146	162	175	179
135	F	I	FI	47	64	100	132	163	167	176	199	214	223
136	F	I	FI	38	52	87	109	152	157	165	179	196	204
137	M	S	MS	44	96	127	164	209	234	287	329	363	376
138	M	S	MS	42	90	123	156	190	235	276	305	332	355
139	M	S	MS	42	106	130	170	203	244	289	320	358	384
140	M	I	MI	42	99	146	182	216	248	295	335	380	394
141	M	I	MI	46	109	166	191	245	282	309	326	411	440
142	M	I	MI	40	111	162	202	232	276	311	354	393	426
143	F	S	FS	33	67	90	114	139	154	167	186	202	204
144	F	S	FS	42	86	118	134	156	172	189	205	224	236
145	F	S	FS	39	72	100	121	137	154	172	197	210	214
146	F	I	FI	42	98	127	136	158	176	204	223	240	248
147	F	I	FI	42	107	141	160	174	196	221	255	277	283
148	F	I	FI	37	104	131	147	165	190	217	231	251	259

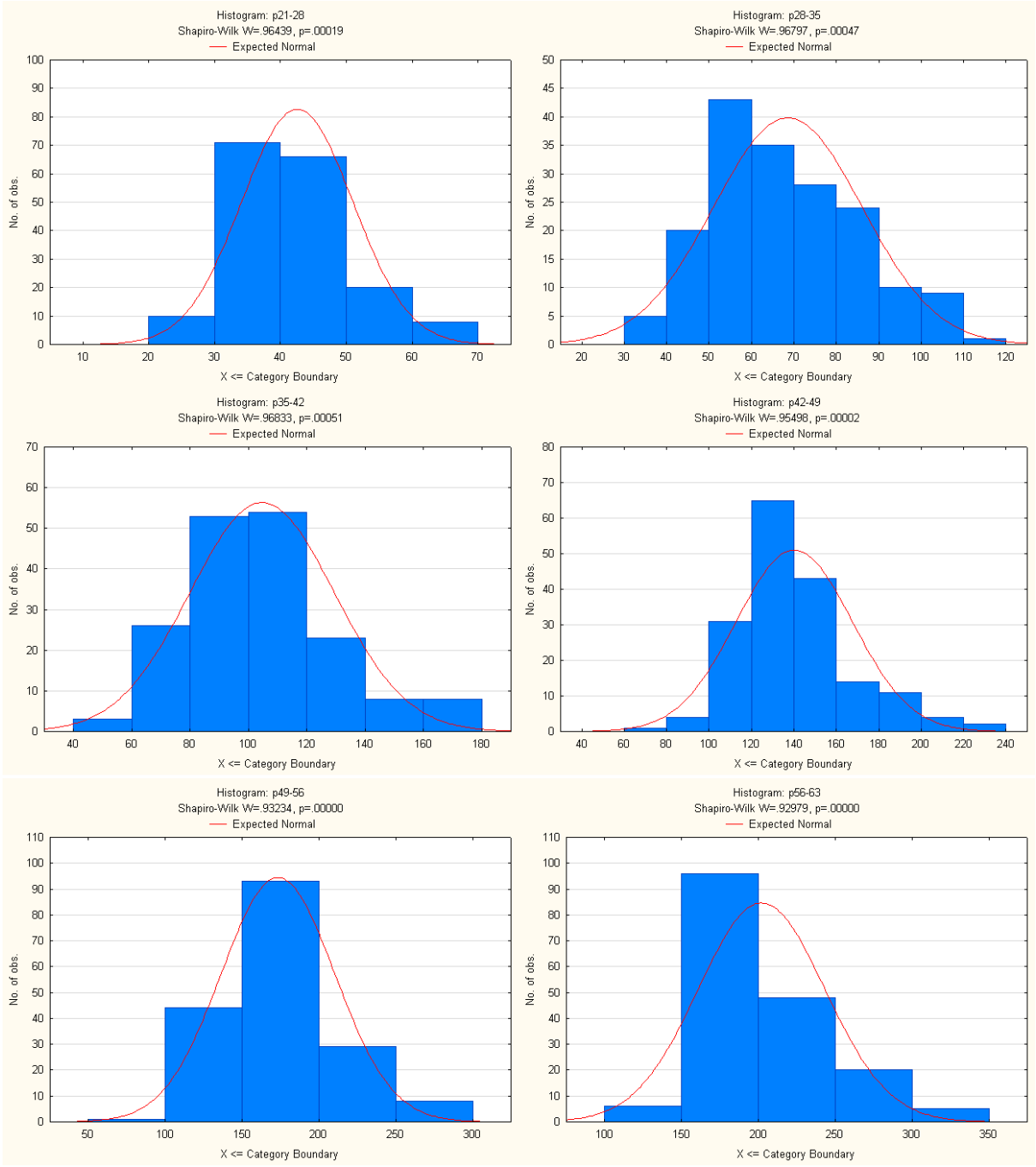


Figure 71- Body weight, Histograms 1/2

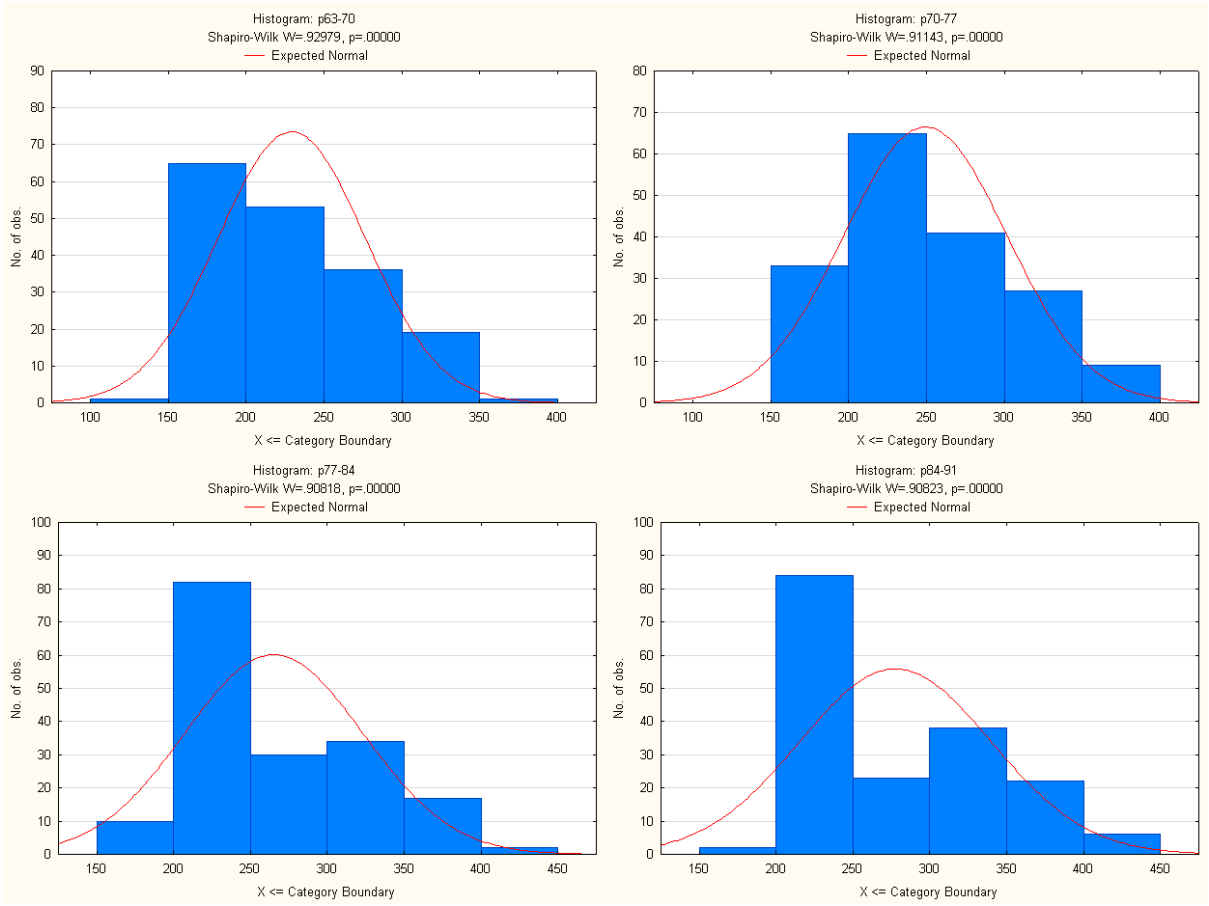


Figure 72- Body weight, Histograms 2/2

Table 13- Body weight, Descriptive statistics

Variable	Sex_Treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
p21-28	FS	50	40.00	28.00	65.00	37.00	47.00
p28-35	FS	50	66.00	38.00	97.00	52.00	75.00
p35-42	FS	50	95.50	56.00	133.00	83.00	108.00
p42-49	FS	50	129.00	74.00	163.00	116.00	134.00
p49-56	FS	50	153.00	93.00	179.00	139.00	163.00
p56-63	FS	50	172.00	126.00	198.00	165.00	180.00
p63-70	FS	50	191.50	155.00	228.00	182.00	201.00
p70-77	FS	50	204.50	186.00	261.00	197.00	210.00
p77-84	FS	50	214.00	197.00	279.00	209.00	224.00
p84-91	FS	50	223.50	203.00	279.00	214.00	236.00
p21-28	FI	49	43.00	28.00	66.00	37.00	47.00
p28-35	FI	49	70.00	40.00	107.00	55.00	80.00
p35-42	FI	49	105.00	70.00	141.00	88.00	119.00
p42-49	FI	49	139.00	101.00	161.00	126.00	151.00
p49-56	FI	49	162.00	122.00	198.00	149.00	168.00
p56-63	FI	49	176.00	141.00	224.00	168.00	187.00
p63-70	FI	49	198.00	146.00	229.00	186.00	214.00
p70-77	FI	49	209.00	162.00	256.00	200.00	227.00
p77-84	FI	49	222.00	175.00	277.00	208.00	240.00
p84-91	FI	49	228.00	179.00	283.00	222.00	246.00
p21-28	MS	38	41.00	29.00	63.00	37.00	44.00
p28-35	MS	38	63.00	47.00	106.00	54.00	74.00
p35-42	MS	38	104.50	62.00	167.00	84.00	120.00
p42-49	MS	38	139.50	87.00	177.00	127.00	154.00
p49-56	MS	38	190.00	104.00	243.00	168.00	205.00
p56-63	MS	38	230.50	137.00	270.00	212.00	237.00
p63-70	MS	38	257.50	179.00	309.00	245.00	280.00
p70-77	MS	38	284.00	216.00	334.00	271.00	302.00
p77-84	MS	38	302.00	256.00	363.00	291.00	321.00
p84-91	MS	38	317.50	280.00	384.00	307.00	340.00
p21-28	MI	38	42.50	23.00	64.00	34.00	48.00
p28-35	MI	38	71.50	34.00	111.00	54.00	96.00
p35-42	MI	38	127.00	60.00	172.00	85.00	150.00
p42-49	MI	38	174.50	107.00	234.00	127.00	193.00
p49-56	MI	38	215.00	134.00	297.00	168.00	242.00
p56-63	MI	38	253.50	190.00	331.00	215.00	276.00
p63-70	MI	38	294.00	230.00	357.00	256.00	311.00
p70-77	MI	38	322.50	253.00	384.00	297.00	335.00
p77-84	MI	38	341.00	268.00	411.00	310.00	367.00
p84-91	MI	38	355.00	282.00	440.00	325.00	388.00

Table 14- Body weight, p21-28 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p21-28 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =1.624866 p =.6538			
Depend.: p21-28	FS R:87.900	FI R:94.735	MS R:81.039	MI R:86.408
FS		1.000000	1.000000	1.000000
FI	1.000000		1.000000	1.000000
MS	1.000000	1.000000		1.000000
MI	1.000000	1.000000	1.000000	

Table 15- Body weight, p28-35 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p28-35 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =5.605760 p =.1325			
Depend.: p28-35	FS R:77.450	FI R:92.082	MS R:83.000	MI R:101.62
FS		0.904778	1.000000	0.159878
FI	0.904778		1.000000	1.000000
MS	1.000000	1.000000		0.655069
MI	0.159878	1.000000	0.655069	

Table 16- Body weight, p35-42 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p35-42 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =15.45129 p =.0015			
Depend.: p35-42	FS R:68.530	FI R:91.592	MS R:86.092	MI R:110.89
FS		0.141256	0.643409	0.000613
FI	0.141256		1.000000	0.467749
MS	0.643409	1.000000		0.197068
MI	0.000613	0.467749	0.197068	

Table 17- Body weight, p42-49 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p42-49 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =30.27370 p =.0000			
Depend.: p42-49	FS R:61.810	FI R:86.439	MS R:90.855	MI R:121.62
FS		0.093540	0.046337	0.000000
FI	0.093540		1.000000	0.007896
MS	0.046337	1.000000		0.048753
MI	0.000000	0.007896	0.048753	

Table 18- Body weight, p49-56 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p49-56 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =58.99136 p =.0000			
Depend.: p49-56	FS R:54.890	FI R:72.255	MS R:110.41	MI R:129.46
FS		0.529019	0.000002	0.000000
FI	0.529019		0.002964	0.000001
MS	0.000002	0.002964		0.606959
MI	0.000000	0.000001	0.606959	

Table 19- Body weight, p56-63 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p56-63 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =109.6512 p =0.000			
Depend.: p56-63	FS R:47.270	FI R:60.388	MS R:121.18	MI R:144.01
FS		1.000000	0.000000	0.000000
FI	1.000000		0.000000	0.000000
MS	0.000000	0.000000		0.297066
MI	0.000000	0.000000	0.297066	

Table 20- Body weight, p63-70 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p63-70 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =125.5444 p =0.000			
Depend.: p63-70	FS R:45.260	FI R:56.908	MS R:126.46	MI R:145.87
FS		1.000000	0.000000	0.000000
FI	1.000000		0.000000	0.000000
MS	0.000000	0.000000		0.569726
MI	0.000000	0.000000	0.569726	

Table 21- Body weight, p70-77 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p70-77 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =129.1544 p =0.000			
Depend.: p70-77	FS R:44.760	FI R:56.327	MS R:127.08	MI R:146.66
FS		1.000000	0.000000	0.000000
FI	1.000000		0.000000	0.000000
MS	0.000000	0.000000		0.552464
MI	0.000000	0.000000	0.552464	

Table 22- Body weight, p77-84 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p77-84 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =130.8186 p =0.000			
Depend.: p77-84	FS R:45.530	FI R:54.827	MS R:128.00	MI R:146.66
FS		1.000000	0.000000	0.000000
FI	1.000000		0.000000	0.000000
MS	0.000000	0.000000		0.650574
MI	0.000000	0.000000	0.650574	

Table 23- Body weight, p84-91 statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); p84-91 (WEIGHT final) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 175) =131.4898 p =0.000			
Depend.: p84-91	FS R:45.520	FI R:54.612	MS R:128.17	MI R:146.78
FS		1.000000	0.000000	0.000000
FI	1.000000		0.000000	0.000000
MS	0.000000	0.000000		0.656572
MI	0.000000	0.000000	0.656572	

A.2 NOVEL OBJECT RECOGNITION TEST

A.2.1 PHASE 1- OPEN FIELD EXPLORATION ANALYSIS

Table 24- NOR P.1, Data 1/2

Rat #	Sex	Treatment	Sex_Treatment	1m Distance (cm)	1m Inner-zone (s)	1m Inner-zone (n)	5m Distance (cm)	5m Inner-zone (s)	5m Inner-zone (n)	10m Distance (cm)	10m Inner-zone (s)	10m Inner-zone (n)
2	F	S	FS	658.18	3.00	4.00	2874.95	33.40	21.00	5348.00	59.00	38.00
3	F	S	FS	646.45	7.00	5.00	3079.24	35.80	23.00	5224.95	69.60	42.00
4	F	S	FS	559.94	8.60	4.00	2754.46	48.80	29.00	5003.29	127.60	55.00
6	F	I	FI	582.67	3.00	4.00	2662.00	18.20	12.00	4683.42	84.60	33.00
7	F	I	FI	597.14	8.00	3.00	2699.24	39.40	28.00	4852.67	75.40	48.00
8	F	I	FI	487.89	3.80	2.00	2534.20	31.60	22.00	4542.25	60.20	40.00
9	M	S	MS	410.64	12.80	6.00	2416.19	40.40	26.00	4374.88	71.60	49.00
10	M	S	MS	388.76	2.80	4.00	2022.56	27.20	24.00	3516.45	39.80	38.00
11	M	S	MS	368.55	5.00	6.00	2371.25	33.00	21.00	4507.29	90.40	50.00
12	M	S	MS	488.81	18.40	5.00	2708.61	60.20	30.00	4694.60	123.60	57.00
13	M	I	MI	431.54	1.80	3.00	2914.61	45.60	42.00	5362.14	78.80	74.00
14	M	I	MI	421.17	4.00	6.00	2500.07	13.60	14.00	4667.46	42.80	41.00
15	M	I	MI	432.07	5.80	3.00	2617.74	46.60	28.00	4558.15	71.40	39.00
16	M	I	MI	510.08	7.80	2.00	3177.99	22.00	23.00	6314.38	55.80	53.00
17	F	S	FS	702.69	3.80	2.00	3839.24	49.00	21.00	6259.44	87.00	45.00
18	F	S	FS	548.36	2.60	2.00	2991.62	56.40	27.00	5828.30	113.00	55.00
19	F	S	FS	467.81	11.20	5.00	2833.17	28.20	19.00	4900.42	51.40	33.00
20	F	S	FS	513.16	0.00	0.00	1566.10	15.00	9.00	2874.96	31.00	18.00
21	F	I	FI	267.50	8.60	2.00	2583.56	21.00	13.00	5116.45	48.20	27.00
22	F	I	FI	342.59	1.20	2.00	2405.99	17.00	11.00	4912.39	47.20	32.00
23	F	I	FI	464.89	1.60	2.00	2437.97	16.60	11.00	4783.29	65.40	28.00
24	F	I	FI	482.14	7.60	3.00	3035.58	30.00	17.00	5645.01	73.80	31.00
25	M	S	MS	582.79	5.60	2.00	3171.49	30.00	21.00	5549.68	80.60	47.00
26	M	S	MS	543.25	11.40	7.00	2773.43	58.40	30.00	4911.33	128.80	58.00
27	M	S	MS	476.43	2.00	3.00	3301.92	38.00	20.00	5857.86	102.80	45.00
28	M	S	MS	341.80	6.60	3.00	1932.08	47.00	21.00	3133.34	53.60	27.00
29	M	I	MI	424.60	4.60	3.00	3304.29	35.60	25.00	6177.58	99.20	54.00
30	M	I	MI	345.43	5.20	4.00	2921.65	10.60	10.00	5838.09	31.40	21.00
31	M	I	MI	520.63	11.20	4.00	3541.16	53.80	29.00	6946.15	96.60	59.00
32	M	I	MI	384.61	12.00	6.00	3194.42	49.00	33.00	6581.01	120.00	65.00
33	F	S	FS	509.64	2.80	3.00	2738.50	41.00	31.00	5074.48	72.60	46.00
34	F	S	FS	477.29	1.40	2.00	2995.73	17.80	11.00	5417.24	37.40	30.00
35	F	S	FS	714.37	2.20	1.00	3665.95	63.60	37.00	6539.51	110.00	69.00
36	F	S	FS	734.05	14.80	7.00	3293.87	53.00	30.00	5732.57	84.20	47.00
37	F	I	FI	542.14	7.20	2.00	2730.16	38.20	20.00	4863.03	78.20	33.00
38	F	I	FI	454.06	9.60	4.00	2322.12	54.20	23.00	4609.90	112.40	50.00
39	F	I	FI	468.21	1.40	1.00	2874.68	25.60	14.00	5641.58	56.60	29.00
40	F	I	FI	388.69	0.00	0.00	3002.07	39.80	17.00	5489.89	71.00	36.00
41	M	S	MS	520.68	6.80	6.00	2668.35	47.60	30.00	4557.49	90.60	58.00
42	M	S	MS	678.30	9.20	5.00	3429.06	79.60	46.00	5916.45	134.00	69.00
43	M	S	MS	456.80	13.40	3.00	3005.99	45.40	28.00	5119.48	75.20	50.00
44	M	S	MS	476.77	6.20	5.00	2874.60	59.60	30.00	5160.74	109.60	57.00
45	M	I	MI	471.14	1.80	3.00	2652.82	29.40	12.00	5536.46	84.00	31.00
46	M	I	MI	456.33	18.80	10.00	2915.88	46.80	31.00	6172.75	93.40	56.00
47	M	I	MI	663.00	10.00	6.00	3777.29	48.80	31.00	6313.61	73.60	50.00
48	M	I	MI	412.79	12.60	6.00	2979.83	49.40	25.00	5533.77	87.00	56.00
49	F	S	FS	568.34	15.20	5.00	3011.75	53.20	29.00	5713.92	110.20	57.00

Table 25- NOR P.1, Data 2/2

Rat #	Sex	Treatment	Sex_Treatment	1m Distance (cm)	1m Inner-zone (s)	1m Inner-zone (n)	5m Distance (cm)	5m Inner-zone (s)	5m Inner-zone (n)	10m Distance (cm)	10m Inner-zone (s)	10m Inner-zone (n)
50	F	S	FS	534.69	6.80	4.00	3054.57	57.20	28.00	5339.97	91.60	45.00
51	F	S	FS	682.23	4.20	4.00	3255.17	38.40	27.00	5746.07	73.40	50.00
52	F	S	FS	451.79	0.20	1.00	2452.88	24.60	16.00	4682.04	58.00	33.00
53	F	I	FI	546.30	4.20	3.00	3571.54	41.00	24.00	6981.84	79.00	44.00
54	F	I	FI	470.92	3.00	1.00	3136.76	11.80	9.00	5722.92	77.20	29.00
56	F	I	FI	393.58	9.00	5.00	2989.24	25.00	17.00	6362.54	52.60	40.00
57	M	S	MS	421.53	11.00	7.00	2436.84	97.80	29.00	4536.37	201.80	59.00
58	M	S	MS	558.09	7.20	4.00	3343.22	56.80	28.00	5594.34	110.40	57.00
59	M	S	MS	404.38	12.40	10.00	2864.37	60.20	34.00	5089.80	108.00	60.00
60	M	S	MS	441.54	3.60	3.00	2736.05	43.00	27.00	4815.92	87.00	51.00
61	M	I	MI	515.87	11.80	4.00	2885.38	58.20	29.00	5721.90	130.80	69.00
62	M	I	MI	472.73	9.80	4.00	2676.77	21.40	16.00	5076.46	48.80	28.00
63	M	I	MI	482.45	0.00	0.00	3192.21	33.40	32.00	6449.55	67.00	65.00
64	M	I	MI	545.78	11.80	5.00	2940.46	41.20	31.00	5536.28	97.60	58.00
65	F	S	FS	78.94	0.00	0.00	2082.76	6.20	7.00	4661.13	24.40	21.00
66	F	S	FS	579.47	2.00	2.00	3476.98	14.20	11.00	6158.92	48.40	32.00
67	F	S	FS	591.05	2.60	2.00	3309.09	27.00	20.00	5718.35	53.00	40.00
68	F	S	FS	576.93	4.80	2.00	3448.31	39.00	25.00	5873.49	109.00	46.00
69	F	I	FI	603.46	13.40	5.00	4152.84	48.20	27.00	7828.75	95.80	61.00
70	F	I	FI	503.04	11.60	3.00	3391.32	58.80	30.00	5868.93	115.20	65.00
71	F	I	FI	577.98	1.60	2.00	3238.87	36.60	27.00	6320.84	89.20	64.00
72	F	I	FI	472.71	7.80	3.00	3369.83	32.60	25.00	6167.54	72.00	43.00
73	M	S	MS	576.55	3.40	4.00	2832.97	24.20	18.00	5460.85	92.80	47.00
74	M	S	MS	553.58	3.00	4.00	3401.40	35.20	22.00	5809.99	88.00	42.00
75	M	S	MS	440.37	7.80	4.00	3138.39	76.00	43.00	5995.45	139.40	75.00
76	M	S	MS	386.08	16.20	6.00	3002.28	64.20	32.00	5520.21	160.80	70.00
77	M	I	MI	374.27	16.40	5.00	2916.36	86.80	39.00	5634.04	137.80	70.00
78	M	I	MI	394.52	23.80	6.00	2947.04	79.80	35.00	5525.67	152.40	73.00
79	M	I	MI	226.25	34.20	4.00	2377.75	91.20	28.00	4439.85	139.60	56.00
80	M	I	MI	275.79	23.60	6.00	2377.26	92.40	43.00	5009.79	159.20	71.00
81	F	S	FS	543.14	11.60	5.00	3206.93	40.40	35.00	6306.46	120.80	69.00
82	F	S	FS	609.95	3.60	3.00	2978.52	30.80	23.00	5216.61	74.60	45.00
83	F	S	FS	518.17	9.00	4.00	3403.98	42.00	47.00	5899.18	92.80	75.00
84	F	S	FS	537.97	5.40	2.00	3045.50	48.00	28.00	5686.41	96.20	52.00
85	F	I	FI	569.98	10.00	4.00	2893.03	65.40	25.00	5337.36	121.20	59.00
86	F	I	FI	552.49	8.40	3.00	2852.38	55.00	28.00	5691.01	106.40	50.00
87	F	I	FI	312.44	0.80	1.00	2158.28	20.80	13.00	4160.53	42.20	25.00
88	F	I	FI	389.03	23.00	7.00	3351.78	88.00	50.00	6059.76	171.00	90.00
89	M	S	MS	608.38	7.80	4.00	2837.42	46.80	30.00	4940.93	117.60	58.00
90	M	S	MS	324.94	6.40	5.00	2369.66	66.00	36.00	4549.91	142.20	73.00
91	M	S	MS	510.36	10.20	6.00	2443.79	60.20	26.00	4032.82	112.80	41.00
92	M	I	MI	328.02	4.80	2.00	2605.47	59.40	27.00	4751.04	126.60	53.00
93	M	I	MI	450.37	17.00	11.00	2788.78	86.40	48.00	4909.16	124.00	73.00
94	M	I	MI	369.21	31.80	9.00	2373.61	97.60	37.00	4756.35	157.60	72.00
95	M	S	MS	299.46	7.60	5.00	2655.17	30.40	21.00	5064.06	57.80	35.00
96	M	S	MS	213.19	12.00	4.00	2940.38	24.00	15.00	5959.69	65.20	42.00
97	M	I	MI	406.35	13.00	3.00	2240.16	40.40	31.00	4283.28	103.40	69.00
98	M	I	MI	411.10	4.40	3.00	2302.86	45.80	27.00	4439.29	88.40	63.00
99	F	S	FS	596.93	11.60	9.00	3100.10	77.40	38.00	5669.00	142.20	68.00
100	F	I	FI	370.27	19.60	6.00	2667.00	56.80	28.00	4533.77	114.40	43.00

A.2.1.1 P.1- FIRST MINUTE

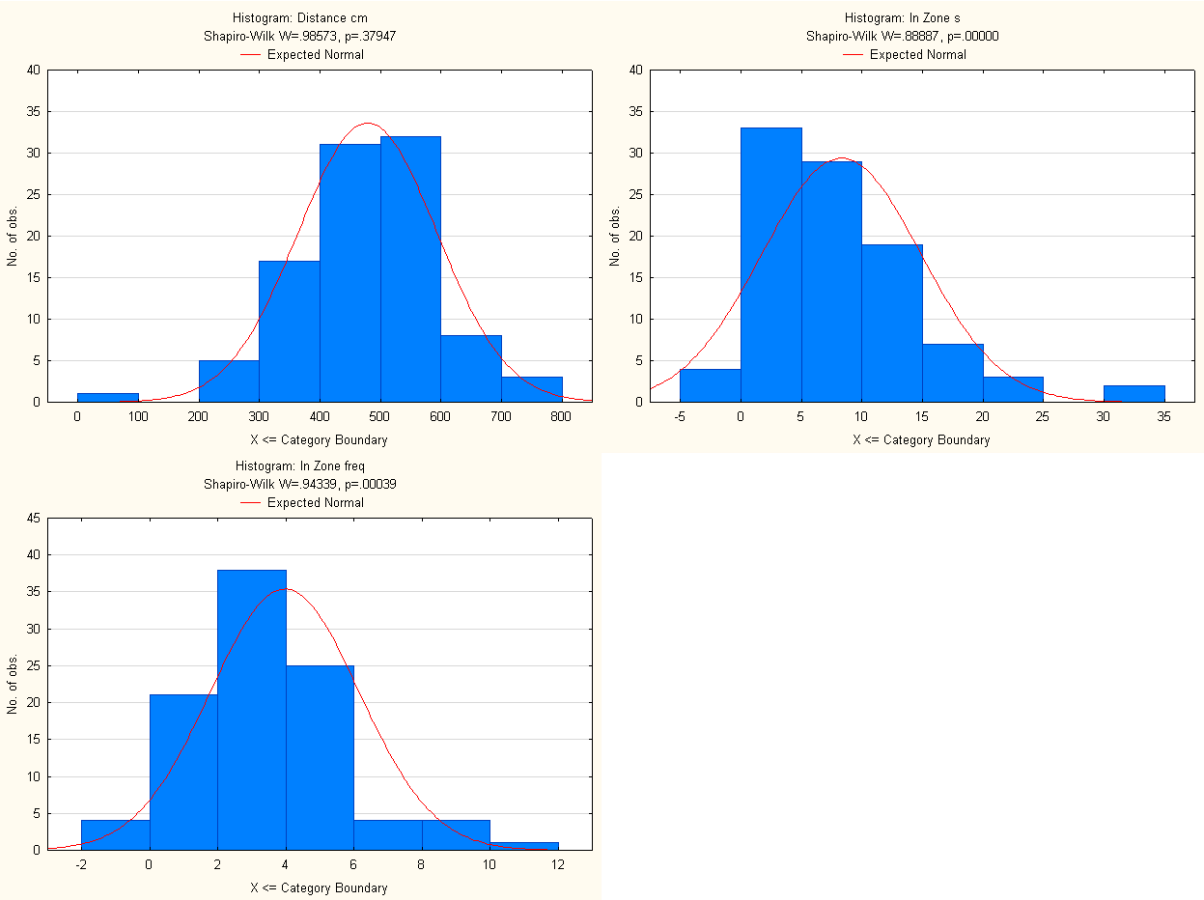


Figure 73- NOR P.1 1 MIN, Histograms

Table 26- NOR P.1 1 MIN, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
Distance cm	F	S	24	558.3969	78.93856	734.0543	128.2879
Distance cm	F	I	23	471.3097	267.5007	603.4645	95.10472
Distance cm	M	S	25	458.8805	213.1915	678.3027	107.3858
Distance cm	M	I	25	429.0442	226.2511	663.0010	89.29005

Variable	Sex_Treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
In Zone s	FS	24	4.0000	0.00000	15.2000	2.4000	8.8000
In Zone freq	FS	24	3.0000	0.00000	9.0000	2.0000	4.5000
In Zone s	FI	23	7.6000	0.0000	23.0000	1.6000	9.6000
In Zone freq	FI	23	3.0000	0.0000	7.0000	2.0000	4.0000
In Zone s	MS	25	7.6000	2.0000	18.4000	5.6000	11.4000
In Zone freq	MS	25	5.0000	2.0000	10.0000	4.0000	6.0000
In Zone s	MI	25	11.2000	0.0000	34.2000	4.8000	16.4000
In Zone freq	MI	25	4.0000	0.0000	11.0000	3.0000	6.0000

Table 27- NOR P.1 1 MIN, Distance travelled (cm) statistics

Parametric factorial ANOVA for all sex and housing groups. Followed by Bonferroni post hoc.

Univariate Tests of Significance for Distance cm (2.1 P1 NOR (1 min)) Sigma-restricted parameterization Effective hypothesis decomposition					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	22267167	1	22267167	1980.490	0.000000
Sex	121724	1	121724	10.826	0.001414
Treatment	82783	1	82783	7.363	0.007935
Sex*Treatment	19847	1	19847	1.765	0.187222
Error	1045623	93	11243		

Bonferroni test; variable Distance cm (2.1 P1 NOR (1 min)) Probabilities for Post Hoc Tests Error: Between MS = 11243., df = 93.000			
Cell No.	Sex	{1} 515.78	{2} 443.96
1	F		0.001231
2	M	0.001231	

Bonferroni test; variable Distance cm (2.1 P1 NOR (1 min)) Probabilities for Post Hoc Tests Error: Between MS = 11243., df = 93.000			
Cell No.	Treatment	{1} 507.62	{2} 449.30
1	S		0.008042
2	I	0.008042	

Table 28- NOR P.1 1 MIN, Time spent in the inner-zone (s) statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

Multiple Comparisons p values (2-tailed); In Zone s (2.1 P1 NOR (1 min)) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 97) =10.61324 p =.0140				
Depend.: In Zone s	FS R:36.875	FI R:43.674	MS R:53.260	MI R:61.280
FS		1.000000	0.249831	0.014469
FI	1.000000		1.000000	0.182303
MS	0.249831	1.000000		1.000000
MI	0.014469	0.182303	1.000000	

Table 29- NOR P.1 1 MIN, Number of inner-zone entries statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

Depend.: In Zone freq	Multiple Comparisons p values (2-tailed); In Zone freq (2.1 P1 NOR (1 min)) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 97) =17.00052 p =.0007 FS R:39.250	FI R:35.174	MS R:62.760	MI R:57.320
FS		1.000000	0.020804	0.147990
FI	1.000000		0.004158	0.038775
MS	0.020804	0.004158		1.000000
MI	0.147990	0.038775	1.000000	

A.2.1.2 P.1- FIVE MINUTES

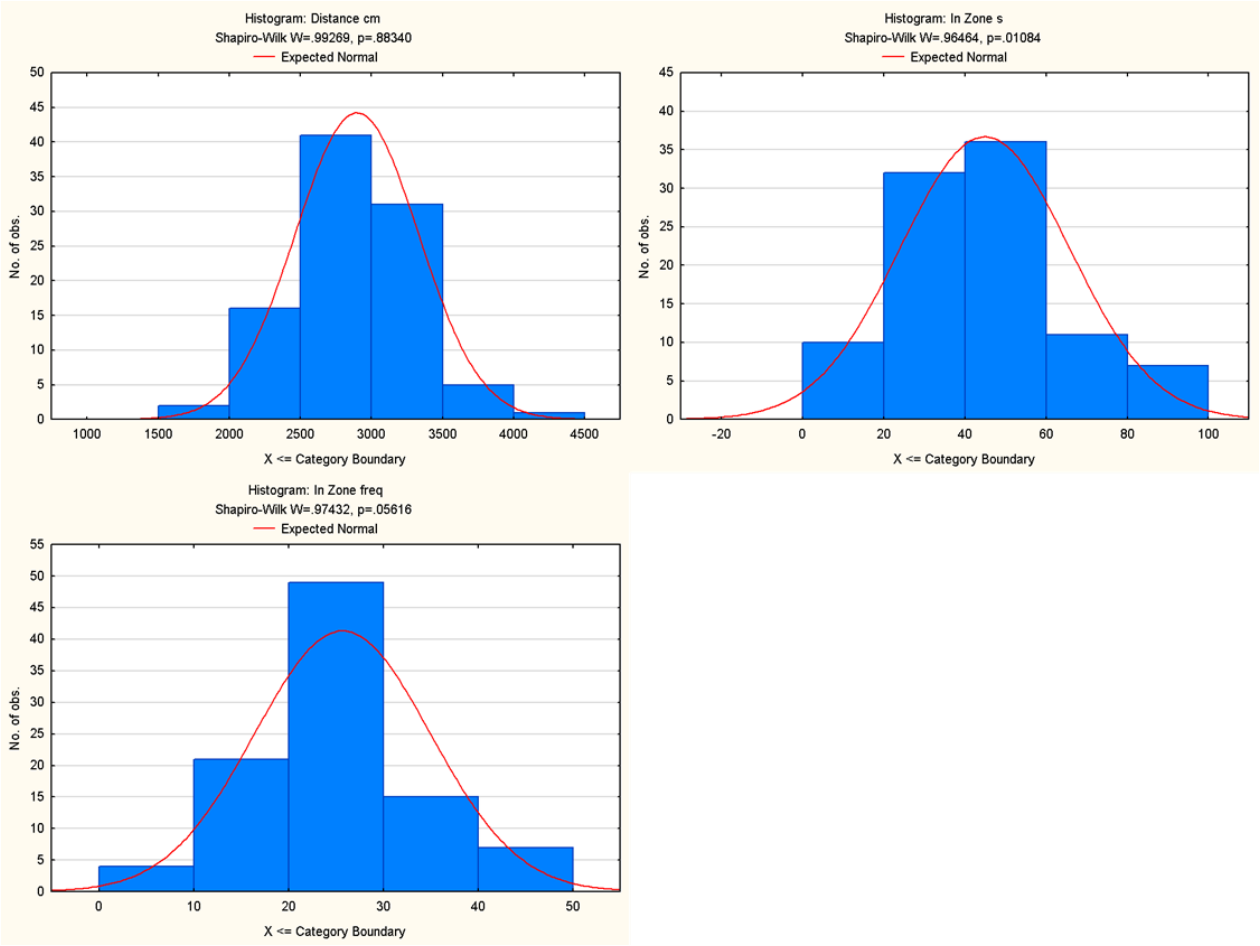


Figure 74- NOR P.1 5 MIN, Histograms

Table 30- NOR P.1 5 MIN, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
Distance cm	F	S	24	3019.14	1566.10	3839.24	485.058
In Zone freq	F	S	24	24.67	7.00	47.00	9.707
Distance cm	F	I	23	2915.67	2158.28	4152.84	459.615
In Zone freq	F	I	23	21.35	9.00	50.00	9.133
Distance cm	M	S	25	2787.10	1932.08	3429.06	400.638
In Zone freq	M	S	25	27.52	15.00	46.00	7.275
Distance cm	M	I	24	2870.07	2302.86	3777.29	373.758
In Zone freq	M	I	24	28.96	10.00	48.00	9.521

Variable	Sex_Treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
In Zone s	FS	24	39.70	6.200	77.40	27.60	51.00
In Zone s	FI	23	36.60	11.80	88.00	21.00	54.20
In Zone s	MS	25	47.00	24.00	97.80	35.20	60.20
In Zone s	MI	24	47.80	10.60	97.60	34.50	69.60

Table 31- NOR P.1 5 MIN, Distance travelled (cm) statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for Distance cm (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	805543932	1	805543932	4327.816	0.000000
Sex	462107	1	462107	2.483	0.118538
Treatment	2518	1	2518	0.014	0.907655
Sex*Treatment	208379	1	208379	1.120	0.292791
Error	17124122	92	186132		

Table 32- NOR P.1 5 MIN, Time spent in the inner-zone (s) statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups

Depend.: In Zone s	Multiple Comparisons p values (2-tailed); In Zone s (Spreadsheet1) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 96) =7.730323 p =.0519			
	FS R:42.229	FI R:38.761	MS R:56.600	MI R:55.667
FS		1.000000	0.426247	0.568322
FI	1.000000		0.159968	0.225253
MS	0.426247	0.159968		1.000000
MI	0.568322	0.225253	1.000000	

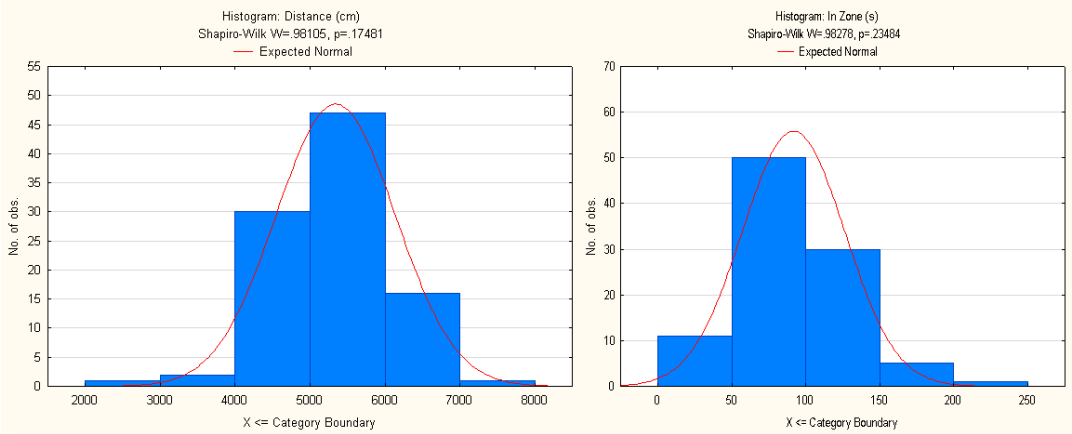
Table 33- NOR P.1 5 MIN, Number of inner-zone entries statistics

Parametric factorial ANOVA for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Univariate Tests of Significance for ln Zone freq (Spreadsheet1) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	62973.92	1	62973.92	787.4148	0.000000
Sex	656.38	1	656.38	8.2073	0.005169
Treatment	21.20	1	21.20	0.2651	0.607890
Sex*Treatment	135.67	1	135.67	1.6963	0.196017
Error	7357.75	92	79.98		

Cell No.	Bonferroni test; variable ln Zone freq (2 NOR 5Ms P1) Probabilities for Post Hoc Tests Error: Between MS = 79.976, df = 92.000 Sex	{1} 23.043	{2} 28.224
1	F		0.005584
2	M	0.005584	

A.2.1.3 P.1- TEN MINUTES



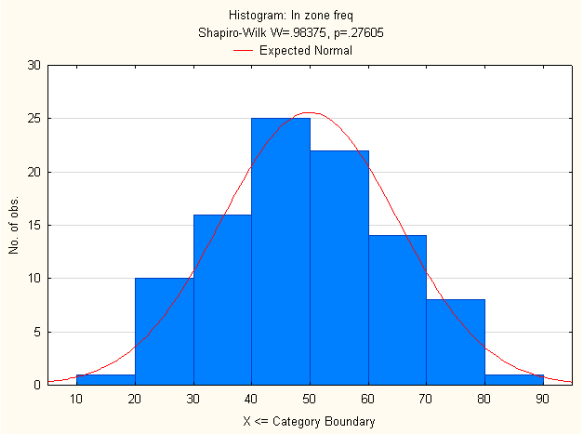


Figure 75- NOR P.1 10 MIN, Histograms

Table 34- NOR P.1 10 MIN, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
Distance (cm)	F	S	24	5453.113	2874.956	6539.511	741.0207
In Zone (s)	F	S	24	80.725	24.400	142.200	31.7390
In zone freq	F	S	24	46.292	18.000	75.000	14.7956
Distance (cm)	F	I	23	5485.899	4160.535	7828.755	879.4618
In Zone (s)	F	I	23	83.009	42.200	171.000	30.0990
In zone freq	F	I	23	43.478	25.000	90.000	15.8999
Distance (cm)	M	S	25	4986.798	3133.337	5995.451	745.4397
In Zone (s)	M	S	25	103.376	39.800	201.800	36.2333
In zone freq	M	S	25	52.600	27.000	75.000	11.9338
Distance (cm)	M	I	25	5461.368	4283.278	6946.147	757.1467
In Zone (s)	M	I	25	98.688	31.400	159.200	36.1670
In zone freq	M	I	25	56.760	21.000	74.000	14.9060

Table 35- NOR P.1 10 MIN, Distance travelled (cm) statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for Distance (cm) (Spreadsheet3) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	2.769757E+09	1	2.769757E+09	4539.756	0.000000
Sex	1.458900E+06	1	1.458900E+06	2.391	0.125416
Treatment	1.558700E+06	1	1.558700E+06	2.555	0.113353
Sex*Treatment	1.181828E+06	1	1.181828E+06	1.937	0.167307
Error	5.674037E+07	93	6.101116E+05		

Table 36- NOR P.1 10 MIN, Time spent in the inner-zone (s) statistics

Parametric factorial ANOVA for all sex and housing groups. Followed by Bonferroni post hoc.

Univariate Tests of Significance for ln Zone (s) (Spreadsheet3) Sigma-restricted parameterization Effective hypothesis decomposition					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	810245.6	1	810245.6	710.8621	0.000000
Sex	8896.5	1	8896.5	7.8053	0.006326
Treatment	35.0	1	35.0	0.0307	0.861270
Sex*Treatment	294.3	1	294.3	0.2582	0.612553
Error	106002.1	93	1139.8		

Bonferroni test; variable ln Zone (s) (2 NOR 10Ms P1) Probabilities for Post Hoc Tests Error: Between MS = 1139.8, df = 93.000			
Cell No.	Sex	{1} 81.843	{2} 101.03
1	F		0.006256
2	M	0.006256	

Table 37- NOR P.1 10 MIN, Number of inner-zone entries statistics

Parametric factorial ANOVA for all sex and housing groups. Followed by Bonferroni post hoc.

Univariate Tests of Significance for ln zone freq (2 NOR 10Ms P1) Sigma-restricted parameterization Effective hypothesis decomposition					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	240108.7	1	240108.7	1154.174	0.000000
Sex	2323.8	1	2323.8	11.170	0.001198
Treatment	11.0	1	11.0	0.053	0.818799
Sex*Treatment	294.5	1	294.5	1.415	0.237186
Error	19347.3	93	208.0		

Bonferroni test; variable ln zone freq (2 NOR 10Ms P1) Probabilities for Post Hoc Tests Error: Between MS = 208.04, df = 93.000			
Cell No.	Sex	{1} 44.915	{2} 54.680
1	F		0.001237
2	M	0.001237	

A.2.2 PHASE 2- OBJECT FAMILIARISATION ANALYSIS

Table 38- NOR P.2, Data 1/2

Rat #	Sex	Treatment	Sex_Treatment	1m Distance (cm)	1m Q1 + Q2 (s)	1m Q3 + Q4 (s)	1m Q1 (s) Raw	1m Q1 (s) Corrected	1m Q2 (s)	1m Q2 freq	1m O1 (s) Raw	1m O1 (s) Corrected	1m O2 (s)	1m O1 freq	1m O1 freq Corrected	1m O2 freq	1m O1 lat (s)	1m O2 lat (s)	1m Q2/Q1 (s) C.Fact	1m O2/O1 (s) C.Fact	1m O2/O1 freq C.Fact	5m Distance (cm)	5m Q1 + Q2 (s)	5m Q3 + Q4 (s)	5m Q1 (s) Raw	5m Q1 (s) Corrected	5m Q2 (s)	5m Q2 freq	5m O1 (s) Raw	5m O1 (s) Corrected	5m O2 (s)	5m O2 freq	5m Q2/Q1 (s) C.Fact	5m O2/O1 (s) C.Fact	5m O2/O1 freq C.Fact
2 F	S	FS		723.66	34.20	25.80	17.60	16.60	16.60	3.00	4.00	8.80	3.00	14.00	11.00	11.00	5.20	0.00	0.94	0.34	0.79	2823.36	199.80	100.20	87.00	112.80	112.80	17.00	17.00	30.00	36.80	36.80	58.00	58.00	1.30	1.23	1.45
3 F	S	FS		871.95	33.20	26.80	13.00	20.20	20.20	4.00	4.00	7.20	8.00	8.00	6.00	12.00	12.00	2.60	4.60	1.55	1.11	2.00	3536.94	214.80	85.20	94.80	120.00	120.00	19.00	22.00	43.80	58.80	58.80	44.00	58.00	58.00	1.27	1.34	1.32
4 F	S	FS		773.98	32.00	28.00	16.80	15.20	15.20	4.00	4.00	6.20	7.20	7.20	8.00	9.00	9.00	0.60	5.40	0.90	1.16	1.13	2938.65	189.00	111.00	96.40	92.60	92.60	17.00	19.00	35.20	36.40	36.40	36.00	43.00	43.00	0.96	1.03	1.19
6 F	I	FI		466.38	25.20	34.80	3.60	21.60	21.60	2.00	4.00	1.00	8.00	8.00	3.00	6.00	6.00	19.80	26.80	6.00	8.00	2.00	2606.49	161.20	138.80	66.80	94.40	94.40	11.00	16.00	23.00	28.00	26.00	35.00	35.00	1.41	1.22	1.35
7 F	I	FI		722.69	31.40	28.60	19.00	12.40	12.40	5.00	4.00	11.60	5.20	5.20	10.00	10.00	10.00	0.00	7.40	0.65	0.45	1.00	2690.42	196.60	103.40	119.60	77.00	77.00	14.00	14.00	65.60	32.60	32.60	51.00	42.00	42.00	0.64	0.50	0.82
8 F	I	FI		790.45	35.00	25.00	14.40	20.60	20.60	3.00	6.00	5.00	6.60	6.60	9.00	10.00	10.00	2.20	3.80	1.43	1.32	1.11	3032.70	180.40	119.60	79.60	100.80	100.80	14.00	18.00	39.00	44.00	44.00	33.00	43.00	43.00	1.27	1.13	1.30
9 M	S	MS		596.36	18.40	41.60	11.00	7.40	7.40	3.00	2.00	3.40	4.00	4.00	4.00	7.00	7.00	11.40	0.20	0.67	1.18	1.75	3213.00	144.80	155.20	81.20	63.60	63.60	18.00	19.00	25.60	32.00	32.00	37.00	30.00	30.00	0.78	1.25	0.81
10 M	S	MS		554.89	29.60	30.40	19.80	9.80	9.80	3.00	2.00	9.20	5.80	5.80	9.00	5.00	5.00	1.60	6.60	0.49	0.63	0.56	2390.53	171.40	128.60	89.60	81.80	81.80	16.00	14.00	42.20	33.20	33.20	33.00	33.00	33.00	0.91	0.79	1.00
11 M	S	MS		568.78	30.00	30.00	22.00	8.00	8.00	2.00	2.00	12.00	3.20	3.20	7.00	4.00	4.00	8.40	0.00	0.36	0.27	0.57	2289.35	153.00	147.00	83.80	69.20	69.20	10.00	11.00	41.60	35.60	35.60	25.00	30.00	30.00	0.83	0.86	1.20
12 M	S	MS		600.71	38.20	21.80	16.40	21.80	21.80	3.00	3.00	5.40	7.80	7.80	5.00	10.00	10.00	8.00	1.20	1.33	1.44	2.00	2665.78	171.60	128.40	90.00	81.60	81.60	13.00	12.00	37.40	34.60	34.60	33.00	32.00	32.00	0.91	0.93	0.97
13 M	I	MI		659.83	25.40	34.60	14.40	11.00	11.00	4.00	2.00	5.40	7.80	7.80	8.00	7.00	7.00	1.60	10.80	0.76	1.44	0.88	2862.48	165.60	134.40	73.00	92.60	92.60	15.00	15.00	26.60	45.80	45.80	30.00	39.00	39.00	1.27	1.72	1.30
15 M	I	MI		507.45	44.00	16.00	23.80	20.20	20.20	4.00	3.00	9.60	10.60	10.60	10.00	11.00	11.00	1.20	8.60	0.85	1.10	1.10	2324.21	206.40	93.60	123.60	82.80	82.80	15.00	12.00	50.80	32.60	32.60	51.00	40.00	40.00	0.67	0.64	0.78
16 M	I	MI		751.92	26.80	33.20	13.20	13.60	13.60	4.00	3.00	4.60	7.00	7.00	11.00	7.00	7.00	0.00	9.80	1.03	1.52	0.64	2758.20	181.00	119.00	81.60	99.40	99.40	13.00	15.00	28.40	49.40	49.40	44.00	57.00	57.00	1.22	1.74	1.30
17 F	S	FS		932.21	30.80	29.20	13.80	17.00	17.00	4.00	4.00	6.40	10.20	10.20	8.00	10.00	10.00	6.60	3.40	1.23	1.59	1.25	3553.60	186.80	113.20	97.40	89.40	89.40	23.00	19.00	37.60	29.20	29.20	44.00	42.00	42.00	0.92	0.78	0.95
18 F	S	FS		860.54	32.60	27.40	16.60	16.00	16.00	4.00	5.00	4.40	9.20	9.20	5.00	7.00	7.00	1.40	4.40	0.96	2.09	1.40	3920.67	176.80	123.20	101.40	75.40	75.40	20.00	18.00	33.20	34.20	34.20	39.00	30.00	30.00	0.74	1.03	0.77
19 F	S	FS		506.25	18.20	41.80	11.00	7.20	7.20	2.00	1.00	4.40	5.20	5.20	4.00	1.00	1.00	25.80	18.80	0.65	1.18	0.25	2872.76	160.40	139.60	73.60	84.80	84.80	12.00	12.00	21.60	42.60	42.60	33.00	51.00	51.00	1.12	1.97	1.55
20 F	S	FS		206.87	11.20	48.80	3.40	7.80	7.80	1.00	1.00	1.60	3.20	3.20	2.00	7.00	7.00	1.20	4.60	2.29	2.00	3.50	1749.65	143.60	156.40	65.20	78.40	78.40	9.00	6.00	24.60	27.80	27.80	22.00	26.00	26.00	1.20	1.13	1.18
21 F	I	FI		683.83	41.20	18.80	12.60	28.60	28.60	3.00	3.00	5.20	14.60	14.60	5.00	12.00	12.00	7.80	2.40	2.27	2.81	2.40	3291.35	188.80	111.20	96.00	92.80	92.80	14.00	14.00	30.00	42.40	42.40	35.00	40.00	40.00	0.97	1.41	1.14
23 F	I	FI		821.50	36.60	23.40	22.40	14.20	14.20	4.00	4.00	13.40	5.40	5.40	6.00	10.00	10.00	1.40	7.20	0.63	0.40	1.67	3152.80	172.40	127.60	99.60	72.80	72.80	14.00	16.00	51.60	34.20	34.20	34.00	38.00	38.00	0.73	0.66	1.12
24 F	I	FI		710.26	20.60	39.40	13.40	7.20	7.20	4.00	2.00	6.20	5.20	5.20	8.00	3.00	3.00	0.00	2.20	0.54	0.84	0.38	3100.33	146.20	153.80	79.00	67.20	67.20	15.00	14.00	28.60	33.40	33.40	34.00	28.00	28.00	0.85	1.17	0.82
25 M	S	MS		761.55	31.20	28.80	16.60	14.60	14.60	3.00	4.00	6.40	6.40	6.40	8.00	5.00	5.00	5.20	10.80	0.88	1.00	0.63	2658.50	172.60	127.40	91.20	81.40	81.40	10.00	11.00	53.00	40.80	40.80	34.00	27.00	27.00	0.89	0.77	0.79
26 M	S	MS		611.76	37.60	22.40	12.60	25.00	25.00	2.00	4.00	5.40	10.60	10.60	3.00	7.00	7.00	11.80	0.00	1.98	1.96	2.33	2606.24	208.00	92.00	100.40	107.60	107.60	11.00	13.00	50.00	53.20	53.20	34.00	41.00	41.00	1.07	1.06	1.21
27 M	S	MS		580.59	40.00	20.00	15.40	24.60	24.60	4.00	3.00	6.60	12.80	12.80	6.00	13.00	13.00	19.20	12.20	1.60	1.94	2.17	2567.05	225.60	74.40	106.60	119.00	119.00	15.00	11.00	44.40	69.40	69.40	39.00	54.00	54.00	1.12	1.56	1.38
28 M	S	MS		303.03	60.00	0.00	12.40	47.60	47.60	2.00	2.00	2.20	18.20	18.20	5.00	25.00	25.00	18.80	0.00	3.84	8.27	5.00	3101.83	216.60	83.40	108.00	108.60	108.60	15.00	16.00	39.80	44.60	44.60	40.00	53.00	53.00	1.01	1.12	1.33
29 M	I	MI		419.61	49.80	10.20	23.20	26.60	26.60	3.00	3.00	11.40	12.60	12.60	12.00	9.00	9.00	20.20	0.00	1.15	1.11	0.75	2704.63	208.80	91.20	75.40	133.40	133.40	15.00	16.00	28.80	59.20	59.20	37.00	43.00	43.00	1.77	2.06	1.16
30 M	I	MI		549.67	23.00	37.00	5.40	17.60	17.60	1.00	2.00	2.60	12.00	12.00	4.00	9.00	9.00	45.20	11.40	3.26	4.62	2.25	2463.78	147.80	152.20	71.80	76.00	76.00	10.00	10.00	36.40	43.20	43.20	34.00	51.00	51.00	1.06	1.19	1.50
31 M	I	MI		618.76	37.40	22.60	28.00	9.40	9.40	4.00	3.00	8.00	4.60	4.60	9.00	4.00	4.00	0.00	13.00	0.34	0.58	0.44	2607.48	194.80	105.20	103.20	91.60	91.60	13.00	13.00	41.60	49.00	49.00	41.00	33.00	33.00	0.89	1.18	0.80
32 M	I	MI		526.99	40.60	19.40	19.00	21.60	21.60	3.00	3.00	6.80	14.40	14.40	6.00	8.00	8.00	0.40	5.80	1.14	2.12	1.33	3117.24	203.00	97.00	79.40	123.60	123.60	17.00	14.00	31.00	59.40	59.40	33.00	56.00	56.00	1.36	1.92	1.70
33 F	S	FS		768.31	43.40	16.60	21.00	22.40	22.40	4.00	5.00	4.00	5.40	5.40	9.00	15.00	15.00	2.40	0.80	1.07	1.35	1.67	3213.21	180.80	119.20	79.60	101.20	101.20	20.00	18.00	22.60	39.60	39.60	44.00	61.00	61.00	1.27	1.75	1.39
34 F	S	FS		687.33	35.60	24.40	14.20	21.40	21.40	3.00	3.00	6.80	8.20	8.20	7.00	8.00	8.00	6.60	10.60	1.51	1.21	1.14	3356.63	197.40	102.60	85.40	112.00	112.00	17.00	15.00	31.20	41.80	41.80	40.00	63.00	63.00	1.31	1.34	1.58
35 F	S	FS		784.74	39.80	20.20	14.40	25.40	25.40	5.00	3.00	6.80	11.80	11.80	13.00	18.00	18.00	0.20	9.40	1.76	1.74	1.38	3383.02	196.60	103.40	93.60	103.00	103.00	18.00	14.00	30.60	44.00	44.00	47.00	73.00	73.00	1.10	1.44	1.55
36 F	S	FS		901.61	42.60	17.40	22.60	20.00	20.00	6.00	5.00	10.00	10.00	10.00	21.00	15.00	15.00	3.40	0.00	0.88	1.00	0.71	3713.34	189.40	110.60	96.00	93.40	93.40	21.00	20.00	36.80	38.00	38.00	67.00	64.00	64.00	0.97	1.03	0.96
37 F	I	FI		763.59	36.60	23.40	24.20	12.40	12.40	4.00	3.00	11.00	3.00	3.00	15.00	8.00	8.00	0.20	1.80	0.51	0.27	0.53	3203.70	172.80	127.20	89.00	83.80	83.80	16.00	13.00	28.00	36.40	36.40	46.00	63.00	63.00	0.94	1.30	1.37
38 F	I	FI		821.09	44.80	15.20	27.40	17.40	17.40	4.00	5.00	11.40	7.00	7.00	13.00	14.00	14.00	9.20	4.20	0.64	0.61	1.08	3077.92	156.60	143.40	94.20	62.40	62.40	13.00	14.00	29.00	28.60	28.60	40.00	41.00	41.00	0.66	0.99	1.03
39 F	I	FI		813.46	39.20	20.80	19.40	19.80	19.80	5.00	4.00	8.20	7.80	7.80	11.00	17.00	17.00	0.20	10.40	1.02	0.95	1.55	3098.68	163.80	136.20	92.60	71.20	71.20	15.00	15.00	24.40	16.80	16.80	50.00	46.00	46.00	0.77	0.69	0.92
40 F	I	FI		897.76	34.80	25.20	17.40	17.40	17.40	4.00	5.00	4.60	7.60	7.60	14.00	13.00	13.00	1.00	6.00	1.00	1.65	0.93	3651.95	184.20	115.80	87.00	97.20	97.20	21.00	17.00	29.60	58.20	58.20	59.00	71.00	71.00	1.12	1.97	1.20
41 M	S	MS		529.15	38.40	21.60	27.60	10.80	10.80	3.00	2.00	8.80	4.20	4.20	19.00	4.00	4.00	0.20	10.60	0.39	0.48	0.21	2642.82	190.40	109.60	115.40	75.00	75.00	11.00	11.00	46.20	32.80	32.80	65.00	56.00	56.00	0.65	0.71	0.86
42 M	S	MS		843.79	32.60	27.40	14.60	18.00	18.00	5.00	4.00	4.40	7.60	7.60	11.00	17.00	17.00	1.00	3.20	1.23	1.73	1.55	3732.31	194.20	105.80	89.80	104.40	104.40	18.00	17.00	36.20	53.00	53.00	61.00	85.00	85.00	1.16	1.46	1.39
43 M	S	MS		658.73	29.60	30.40	15.20	14.40	14.40	4.00	3.00	5.20	7.20	7.20	12.00	13.00	13.00	0.00	4.00	0.95	1.38	1.08	2844.02	222.20	77.80	124.60	97.60	97.60	16.00	16.00	60.80	53.00	53.00	86.00	69.00	69.00	0.78	0.87	0.80
44 M	S	MS		793.93	36.60	23.40	27.20	9.40	9.40	4.00	3.00	11.80	4.60	4.60	18.00	9.00	9.00	0.00	8.20	0.35	0.39	0.50	3031.69	185.20	114.80	107.40	77.80	77.80	15.00	12.00	50.20	32.80	32.80	62.00	48.00	48.00	0.72	0.65	0.77
45 M	I	MI		679.12	31.60	28.40	13.20	18.40	18.40	4.00	5.00	3.00	7.80	7.80	9.00	11.00	11.00	6.40	0.00	1.39	2.60	1.22	2659.32	175.60	124.40	84.80	90.80	90.80	15.00	16.00	27.60	26.20	26.20	40.00	43.00	43.00	1.07	0.95	1.08
46 M	I	MI		558.65	40.20	19.80	17.80	22.40	22.40	3.00	4.00	9.80	9.00	9.00	11.00	9.00	9.00	0.40	12.60	1.26	0.92	0.82	2692.46	204.00	96.00	87.60	116.40	116.40	16.00	13.00	43.60	54.00	54.00	47.00	74.00	74.00	1.33	1.24	1.57
47 M	I	MI		536.13	39.60	20.40	19.20	20.40	20.40	3.00	3.00	8.20	8.20	8.20	11.00	21.00	21.00	18.20	0.80	1.06	1.00	1.91	2512.32	163.40	136.60	100.20	63.20	63.20	13.00	11.00	43.60	27.00	27.00	74.00	56.00	56.00	0.63	0.62	0.76
48 M	I	MI		824.25	33.20	26.80	13.40	19.80	19.80	3.00	4.00	4.80	9.20	9.20	11.00	16.00	16.00	8.60	3.60	1.48	1.92	1.45	3161.58	168.60	131.40	84.80	83.80	83.80	17.00	15.00	34.20	30.80	30.80	52.00	53.00	53.00	0.99	0.90	1.02
49 F	S	FS		629.38	39.20	20.80	15.40	23.80	23.80	3.00	4.00	7.60	14.20	14.20	6.00	10.00	10.00	0.80	12.00	1.55	1.87	1.67	3549.61	177.80	122.20	81.40	96.40	96.40	17.00	19.00	32.40	47.00	47.00	45.00	66.00	66.00	1.18	1.45	1.47
50 F	S	FS		736.85	29.60	30.40	20.60	9.00	9.00	4.00	3.00	11.60	4.40	4.40	11.00	10.00	10.00	0.00	9.80	0.44	0.38	0.91	3641.36	208.20	91.80	109.80	98.40	98.40	21.00	16.00	52.80	39.60	39.60	60.00	52.00	52.00	0.90	0.75	0.87
51 F	S	FS		908.76	36.00	24.00	17.60	18.40	18.40	5.00	5.00	7.80	9.20	9.20	14.00	17.00	17.00	10.80	0.20	1.05	1.18	1.21	3288.02	199.80	100.20	109.60	90.20	90.20	16.00	15.00	38.40	48.20	48.20	57.00	60.00	60.00	0.82	1.26	1.05

Table 39- NOR P.2, Data 2/2

Rat #	Sex	Treatment	Sex_Treatment	1m Distance (cm)	1m Q1 + Q2 (s)	1m Q3 + Q4 (s)	1m Q1 (s) Raw	1m Q1 (s) Corrected	1m Q2 (s)	1m Q2 freq	1m O1 (s) Raw	1m O1 (s) Corrected	1m O2 (s)	1m O1 freq	1m O2 freq	1m O1 1st (s)	1m O2 1st (s)	1m Q2 Q1 (s)	1m O2 O1 (s)	1m O2 O1 C.Fact	5m Distance (cm)	5m Q1 + Q2 (s)	5m Q3 + Q4 (s)	5m Q1 (s) Raw	5m Q1 (s) Corrected	5m Q2 (s)	5m Q2 freq	5m O1 (s) Raw	5m O1 (s) Corrected	5m O2 (s)	5m O1 freq Raw	5m O1 freq Corrected	5m O2 freq	5m Q2 Q1 (s)	5m O2 O1 (s)	5m O2 O1 C.Fact
52 F	S	FS	FS	673.03	36.60	23.40	17.40	19.20	19.20	4.00	3.00	8.60	6.80	11.00	12.00	12.00	3.20	0.40	1.10	0.79	1.09	2669.24	199.00	101.00	84.20	114.80	114.80	13.00	13.00	35.40	55.60	55.60	32.00	58.00	58.00	1.36	1.57	1.81
53 F	I	FI	FI	778.61	44.20	15.80	17.00	27.20	27.20	5.00	4.00	7.40	16.60	6.00	19.00	19.00	6.00	0.20	1.60	2.24	3.17	4508.30	197.60	102.40	85.20	112.40	112.40	22.00	22.00	35.80	60.80	60.80	50.00	67.00	67.00	1.32	1.70	1.34
54 F	I	FI	FI	930.64	40.40	19.60	13.00	27.40	27.40	4.00	5.00	4.20	13.60	13.60	16.00	16.00	6.20	0.00	2.11	3.24	1.60	3823.75	208.40	91.60	97.80	110.60	110.60	19.00	20.00	35.00	50.80	50.80	38.00	72.00	72.00	1.13	1.45	1.24
56 F	I	FI	FI	787.96	48.60	11.40	22.40	26.20	26.20	4.00	4.00	9.00	15.20	15.20	16.00	23.00	23.00	0.00	9.60	1.17	1.69	1.44	3658.94	199.40	100.60	142.40	46.20	111.40	111.40	11.00	15.00	18.80	72.20	72.20	34.00	59.00	59.00	2.41	3.84	1.74
57 M	S	MS	MS	626.98	28.40	31.60	11.60	16.80	16.80	3.00	3.00	3.00	8.20	8.20	8.00	12.00	12.00	0.80	4.40	1.45	2.73	1.50	2674.00	157.60	142.40	46.20	111.40	111.40	11.00	15.00	18.80	72.20	72.20	34.00	59.00	59.00	2.41	3.84	1.74
58 M	S	MS	MS	814.31	33.40	26.60	14.60	18.80	18.80	4.00	6.00	5.00	8.60	8.60	8.00	15.00	15.00	5.00	0.00	1.29	1.72	1.88	3102.06	163.40	136.60	62.40	101.00	101.00	15.00	17.00	21.20	43.40	43.40	27.00	68.00	68.00	1.62	1.87	2.52
59 M	S	MS	MS	575.39	43.20	16.80	20.60	22.60	22.60	3.00	4.00	10.00	9.00	9.00	15.00	11.00	11.00	4.40	0.40	1.10	0.90	0.73	2792.64	193.20	106.80	83.00	110.20	110.20	16.00	14.00	34.80	52.00	52.00	44.00	63.00	63.00	1.33	1.49	1.43
60 M	S	MS	MS	715.05	34.80	25.20	18.60	16.20	16.20	5.00	3.00	7.60	7.80	11.00	12.00	12.00	1.40	20.20	0.87	1.03	1.09	2706.97	209.40	90.60	104.80	104.60	104.60	16.00	13.00	41.60	50.40	50.40	38.00	56.00	56.00	1.00	1.21	0.97
61 M	I	MI	MI	524.69	31.60	28.40	6.00	25.60	25.60	2.00	3.00	3.20	14.20	14.20	4.00	20.00	20.00	16.20	0.00	4.27	4.44	5.00	2761.60	138.80	161.20	44.20	94.60	94.60	12.00	12.00	19.40	50.60	50.60	26.00	73.00	73.00	2.14	2.61	2.81
62 M	I	MI	MI	685.26	36.60	23.40	12.40	24.20	24.20	3.00	4.00	4.20	15.80	15.80	7.00	7.00	23.00	4.60	1.95	3.76	1.00	2951.79	188.60	111.40	80.80	107.80	107.80	20.00	17.00	29.60	53.00	53.00	37.00	39.00	39.00	1.33	1.79	1.05
63 M	I	MI	MI	589.77	27.00	33.00	15.20	11.80	11.80	2.00	2.00	6.60	5.40	5.40	7.00	6.00	6.00	21.20	34.20	0.78	0.82	0.86	2941.89	141.20	155.80	78.40	65.80	65.80	13.00	11.00	24.40	21.60	21.60	36.00	33.00	33.00	0.84	0.89	0.92
64 M	I	MI	MI	639.05	33.80	26.20	20.20	13.60	13.60	3.00	4.00	9.00	4.60	4.60	9.00	9.00	9.00	0.00	13.00	0.67	0.51	1.00	2792.10	174.60	125.40	81.40	93.20	93.20	16.00	14.00	35.60	45.60	45.60	32.00	56.00	56.00	1.14	1.28	1.75
65 F	S	FS	FS	614.38	32.60	27.40	15.60	17.00	17.00	2.00	3.00	4.20	6.80	6.80	13.00	9.00	9.00	16.40	0.60	1.09	1.62	0.69	2499.84	169.60	130.40	84.00	85.60	85.60	11.00	12.00	19.60	36.20	36.20	40.00	41.00	41.00	1.02	1.85	1.03
66 F	S	FS	FS	850.63	32.60	27.40	15.20	17.40	17.40	5.00	4.00	8.00	9.40	9.40	9.00	11.00	11.00	16.40	8.40	1.14	1.18	1.22	3275.09	155.00	145.00	76.20	78.80	78.80	17.00	15.00	36.40	36.20	36.20	40.00	33.00	33.00	1.03	0.99	0.83
67 F	S	FS	FS	792.07	33.60	26.40	21.00	12.60	12.60	3.00	4.00	10.60	8.20	8.20	12.00	9.00	9.00	7.00	0.00	0.60	0.77	0.75	3180.63	171.20	128.80	83.60	87.60	87.60	14.00	15.00	41.80	38.60	38.60	50.00	51.00	51.00	1.05	0.92	1.02
68 F	S	FS	FS	840.35	38.40	21.60	20.60	17.80	17.80	4.00	4.00	7.20	7.00	7.00	11.00	8.00	8.00	0.00	8.20	0.86	0.97	0.73	3382.68	208.00	92.00	106.80	101.20	101.20	15.00	16.00	63.20	57.80	57.80	52.00	45.60	45.60	0.95	0.91	0.87
69 F	I	FI	FI	641.84	41.20	18.80	14.40	26.80	26.80	3.00	4.00	5.60	14.40	14.40	11.00	19.00	19.00	15.80	0.00	1.86	2.57	1.73	3479.12	198.00	102.00	88.00	110.00	110.00	18.00	19.00	35.60	54.80	54.80	67.00	85.00	85.00	1.25	1.54	1.27
70 F	I	FI	FI	597.01	44.00	16.00	18.20	25.80	25.80	4.00	4.00	9.00	12.00	12.00	6.00	11.00	11.00	8.20	0.20	1.42	1.33	1.83	3291.86	196.40	103.60	91.00	105.40	105.40	18.00	19.00	48.80	51.00	51.00	46.00	59.00	59.00	1.16	1.05	1.28
71 F	I	FI	FI	786.55	34.00	26.00	16.20	17.80	17.80	4.00	5.00	8.00	10.80	10.80	6.00	8.00	8.00	9.40	0.00	1.10	1.35	1.33	3587.65	161.20	138.80	69.00	92.20	92.20	18.00	20.00	36.20	54.20	54.20	24.00	34.00	34.00	1.34	1.50	1.42
72 F	I	FI	FI	714.33	33.20	26.80	17.40	15.80	15.80	3.00	3.00	5.80	9.00	9.00	13.00	13.00	13.00	2.00	13.60	0.91	1.55	1.00	3629.33	178.40	121.60	80.40	98.00	98.00	16.00	17.00	29.80	49.00	49.00	48.00	59.00	59.00	1.22	1.64	1.23
73 M	S	MS	MS	669.81	34.00	26.00	14.80	19.20	19.20	3.00	5.00	3.60	10.60	10.60	8.00	7.00	7.00	1.80	0.60	1.30	2.94	0.88	2627.35	163.60	136.40	67.00	96.60	96.60	14.00	16.00	33.20	49.00	49.00	37.00	40.00	40.00	1.44	1.48	1.08
74 M	S	MS	MS	703.50	35.80	24.20	20.00	15.80	15.80	4.00	3.00	7.20	6.80	6.80	10.00	6.00	6.00	0.00	5.00	0.79	0.94	0.60	3106.02	168.60	131.40	82.80	85.80	85.80	16.00	21.00	31.20	44.40	44.40	41.00	40.00	40.00	1.04	1.42	0.98
75 M	S	MS	MS	631.96	24.20	35.80	11.20	13.00	13.00	3.00	3.00	5.80	4.00	4.00	5.00	7.00	7.00	11.80	0.40	1.16	0.69	1.40	3249.67	178.00	122.00	79.40	98.60	98.60	15.00	18.00	43.80	52.80	52.80	23.00	55.00	55.00	1.24	1.21	2.39
76 M	S	MS	MS	726.51	45.20	14.80	20.60	24.60	24.60	5.00	5.00	10.00	16.00	16.00	14.00	23.00	23.00	0.00	10.20	1.19	1.63	1.64	2926.71	210.80	89.20	104.20	106.60	106.60	12.00	14.00	46.80	67.20	67.20	50.00	78.00	78.00	1.02	1.44	1.56
77 M	I	MI	MI	558.07	40.40	19.60	14.20	26.20	26.20	3.00	4.00	6.80	17.80	17.80	9.00	10.00	10.00	4.40	0.00	1.85	2.62	1.11	3035.98	217.20	82.80	95.40	121.80	121.80	16.00	14.00	47.00	81.80	81.80	49.00	62.00	62.00	1.28	1.74	1.27
78 M	I	MI	MI	547.58	33.80	26.20	15.00	18.80	18.80	3.00	2.00	5.40	13.60	13.60	5.00	7.00	7.00	0.20	13.60	1.25	2.52	1.40	3026.18	182.80	117.20	80.80	102.00	102.00	19.00	14.00	38.80	61.40	61.40	37.00	55.00	55.00	1.26	1.58	1.49
79 M	I	MI	MI	524.75	35.00	25.00	18.20	16.80	16.80	2.00	3.00	5.00	6.80	6.80	8.00	6.00	6.00	6.40	0.60	0.92	1.36	0.75	2486.81	194.80	105.20	91.00	103.80	103.80	11.00	12.00	44.20	44.80	44.80	47.00	54.00	54.00	1.14	1.01	1.15
80 M	I	MI	MI	537.57	45.80	14.20	21.40	24.40	24.40	4.00	3.00	10.60	9.20	16.00	7.00	7.00	7.00	0.00	11.40	1.14	0.87	0.44	2513.12	222.80	77.20	105.00	117.80	117.80	13.00	14.00	46.60	58.20	58.20	68.00	57.00	57.00	1.12	1.25	0.84
81 F	S	FS	FS	906.32	36.80	23.20	17.40	19.40	19.40	5.00	6.00	5.40	7.40	7.40	9.00	11.00	11.00	0.00	7.40	1.11	1.37	1.22	3655.92	189.60	110.40	82.40	107.20	107.20	20.00	20.00	29.40	38.00	38.00	51.00	68.00	68.00	1.30	1.29	1.33
82 F	S	FS	FS	877.39	31.40	28.60	11.60	19.80	19.80	5.00	4.00	3.00	9.00	9.00	5.00	11.00	11.00	8.80	0.20	1.71	3.00	2.20	2820.75	218.60	81.40	72.80	145.80	145.80	15.00	16.00	16.60	58.60	58.60	29.00	82.00	82.00	2.00	3.53	2.83
83 F	S	FS	FS	755.15	46.20	13.80	21.80	24.40	24.40	4.00	4.00	8.00	11.80	11.80	12.00	24.00	24.00	0.00	4.00	1.12	1.48	2.00	3413.98	210.80	89.20	76.40	134.40	134.40	16.00	16.00	25.20	57.40	57.40	46.00	96.00	96.00	1.76	2.28	2.09
84 F	S	FS	FS	783.07	34.60	25.40	17.60	17.00	17.00	4.00	5.00	4.40	8.00	8.00	7.00	13.00	13.00	7.40	0.00	0.97	1.82	1.86	2958.60	207.60	92.40	71.40	136.20	136.20	14.00	16.00	22.00	61.00	61.00	32.00	60.00	60.00	1.91	2.77	1.88
85 F	I	FI	FI	514.15	33.80	26.20	12.20	21.60	21.60	2.00	3.00	2.20	4.40	4.40	6.00	13.00	13.00	13.40	0.40	1.77	2.00	2.17	2689.28	176.60	123.40	82.00	94.60	94.60	12.00	14.00	20.20	21.20	21.20	47.00	48.00	48.00	1.15	1.05	1.02
86 F	I	FI	FI	812.22	40.20	19.80	13.80	26.40	26.40	4.00	4.00	4.40	7.80	7.80	9.00	18.00	18.00	4.60	9.00	1.91	1.77	2.00	3793.32	192.60	107.40	80.00	112.60	112.60	21.00	19.00	27.00	44.00	44.00	39.00	57.00	57.00	1.41	1.63	1.46
87 F	I	FI	FI	663.20	38.40	21.60	15.40	23.00	23.00	3.00	3.00	3.80	10.60	10.60	7.00	16.00	16.00	0.00	13.40	1.49	2.79	2.29	3011.14	197.20	102.80	90.80	106.40	106.40	15.00	13.00	23.20	40.20	40.20	50.00	58.00	58.00	1.17	1.73	1.16
88 F	I	FI	FI	886.57	39.00	21.00	19.40	19.60	19.60	5.00	5.00	6.40	7.80	7.80	14.00	12.00	12.00	3.80	0.40	1.01	1.22	0.86	3178.29	188.40	111.60	87.60	100.80	100.80	15.00	14.00	37.80	38.00	38.00	62.00	64.00	64.00	1.15	1.01	1.03
89 M	S	MS	MS	631.56	19.80	40.20	6.80	13.00	13.00	2.00	4.00	1.20	4.80	4.80	4.00	12.00	12.00	2.00	0.60	1.91	4.00	3.00	2704.07	106.80	193.20	42.80	64.00	64.00	10.00	15.00	15.80	30.40	30.40	29.00	48.00	48.00	1.50	1.92	1.66
90 M	S	MS	MS	603.94	35.00	25.00	15.00	20.00	20.00	4.00	3.00	4.80	9.00	9.00	10.00	11.00	11.00	0.00	3.20	1.33	1.88	1.10	2244.35	184.20	115.80	79.00	105.20	105.20	12.00	10.00	28.20	47.80	47.80	36.00	57.00	57.00	1.33	1.70	1.02
91 M	S	MS	MS	655.18	33.80	26.20	18.00	15.80	15.80	3.00	3.00	4.40	9.40	9.40	10.00	11.00	11.00	10.80	0.20	0.88	2.14	1.10	2451.30	195.20	104.80	76.00	119.20	119.20	12.00	13.00	32.60	63.40	63.40	44.00	81.00	81.00	1.57	1.94	1.84
92 M	I	MI	MI	492.08	35.00	25.00	12.60	22.40	22.40	3.00	3.00	5.60	6.20	6.20	6.00	18.00	18.00	22.00	12.40	1.78	1.11	3.00	2604.41	195.80	104.20	75.80	120.00	120.00	8.00	7.00	31.40	50.60	50.60	57.00	69.00	69.00	1.58	1.61	1.21
93 M	I	MI	MI	601.47	34.60	25.20	9.40	25.20	25.20	2.00	3.00	4.20	6.60	6.60	6.00	14.00	14.00	2.00	6.80	2.68	1.57	2.33	2569.06	215.20	84.20	80.20	135.00	135.00	11.00	12.00	45.40	63.20	63.20	66.00	80.00	80.00	1.68	1.39	1.21
94 M	I	MI	MI	484.72	31.20	28.80	5.60	25.60	25.60	1.00	3.00	1.80	7.00	7.00	1.00	12.00	12.00	28.00	0.00	4.57	3.89	12.00	2254.20	213.80	85.80	78.00	135.80	135.80	9.00	13.00	41.80	42.80	42.80	37.00	63.00	63.00	1.74	1.02	1.70
95 M	S	MS	MS	537.09	39.00	21.00	16.60	22.40	22.40	2.00	4.00	5.00	8.20	8.20	7.00	13.00	13.00	17.00	0.00	1.35	1.64	1.86	1991.13	183.80	116.20	56.60	127.20	127.20	7.00	11.00	20.80	56.00	56.00	30.00	72.00	72.00	2.25	2.69	2.40
96 M	S	MS	MS	528.78	42.40	17.60	25.40	17.00	17.00	3.00	2.00	8.80	12.20	12.20	14.00	12.00	12.00	4.40	12.20	0.67	1.39	0.86	2292.53	221.60	78.40	108.40	113.20	113.20	15.00	14.00	46.00	55.00	55.00	65.00	63.00	63.00	1.04	1.20	0.97
97 M	I	MI	MI	481.42	40.00	20.00	15.60	24.40	24.40	2.00	3.00	6.40	11.20	11.20	12.00	13.00	13.00	11.60	0.20	1.56	1.75	1.08	2557.31	165.80	134.20	88.40	77.40	77.40	12.00	12.00	31.00	26.20	26.20	62.00	46.00	46.00	0.88	0.85	0.74
98 M	I	MI	MI	640.95	44.40	15.60	20.00	24.40	24.40	4.00	5.00	9.60	9.20	9.20	10.00	13.00	13.00	0.40	10.80	1.22	0.96	1.30	2856.84	167.80	132.20	72.40	95.40	95.40	12.00	15.00	27.80	34.40	34.40	48.00	49.00	49.00	1.32	1.24	1.02
99 F	S	FS	FS	824.58	38.20	21.80	17.40	20.80	20.80	4.00	4.00	8.20	10.80	10.80	8.00	13.00	13.00	0.00	7.20	1.20	1.32	1.63	2996.85	210.60	89.20	102.80	107.80	107.80	15.00	13.00	53.60	55.20	55.20	32.00	50.00	50.00	1.05	1.03	1.56
100 F	I	FI	FI	663.32	31.60	28.40	17.20	14.40	14.40	4.00	3.00	10.80	10.80	10.80	6.00	7.00	7.00	0.00	25.60	0.84	1.00	1.17	2666.16	174.40	125.60	96.80	77.60	77.60	13.00	10.00	44.20	50.20	50.20	34.00	28.00	28.00	0.80	1.14	0.82

Equation 2- Calculation of correction factor for phase 2 NOR variables

The variables from the data sets were corrected to remove equipment bias. Quadrant 1 or Object 1 was made equal to the corresponding Quadrant 2 or Object 2 value respectively for each animal.

Using Quadrant 1 and Quadrant 2 (s) as an example:

$$\frac{Quadrant\ 02\ (s)}{Quadrant\ 1\ (s)} = correction\ factor\ for\ each\ animal.$$

$$Quadrant\ 1\ (s)\ \times correction\ factor\ for\ each\ animal$$

$$\therefore Quadrant\ 1\ (s) = Quadrant\ 2\ (s)$$ – no differences

Statistics were reapplied on these phase 2 corrected data, no differences were evident. The correction factor calculated in phase 2 was also applied to phase 3 corresponding variables. This correction factor was calculated in this way for both 1 minute and 5 minute variables.

Table 40- NOR P.2, Preference summary

The table lists where an overall preference for the one of the quadrants or objects was demonstrated by all groups as evidenced by time spent or how many entries or approaches were made.

	1 minute		**5 minute**	
	Time spent (s)	**Entries/approaches**	**Time spent (s)**	**Entries/approaches**
Quadrant	Q2		Q2	
Object	O2	O2	O2	O2

A.2.2.1 P.2- FIRST MINUTE

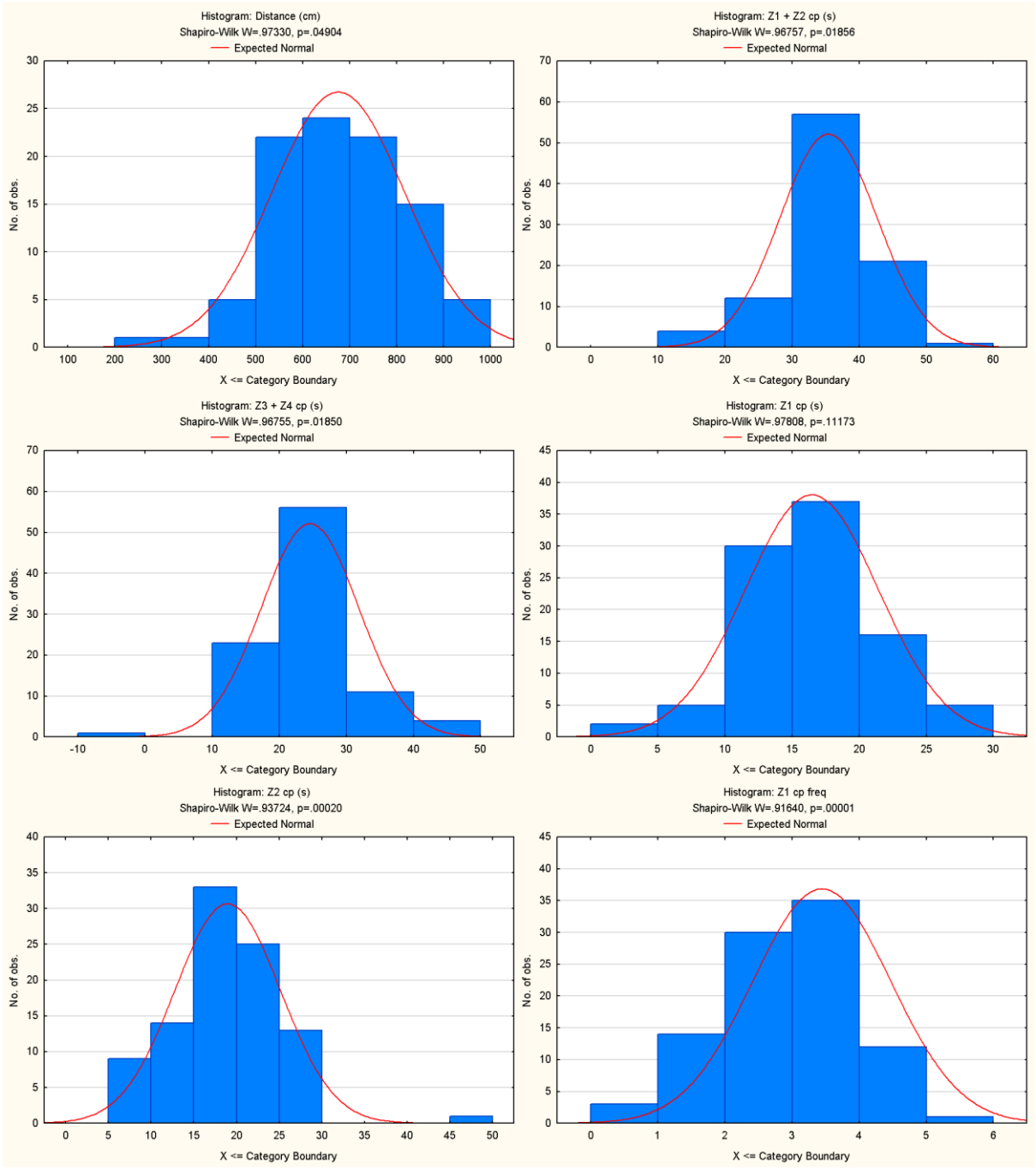


Figure 76- NOR P.2 1 MIN, Histograms 1/3

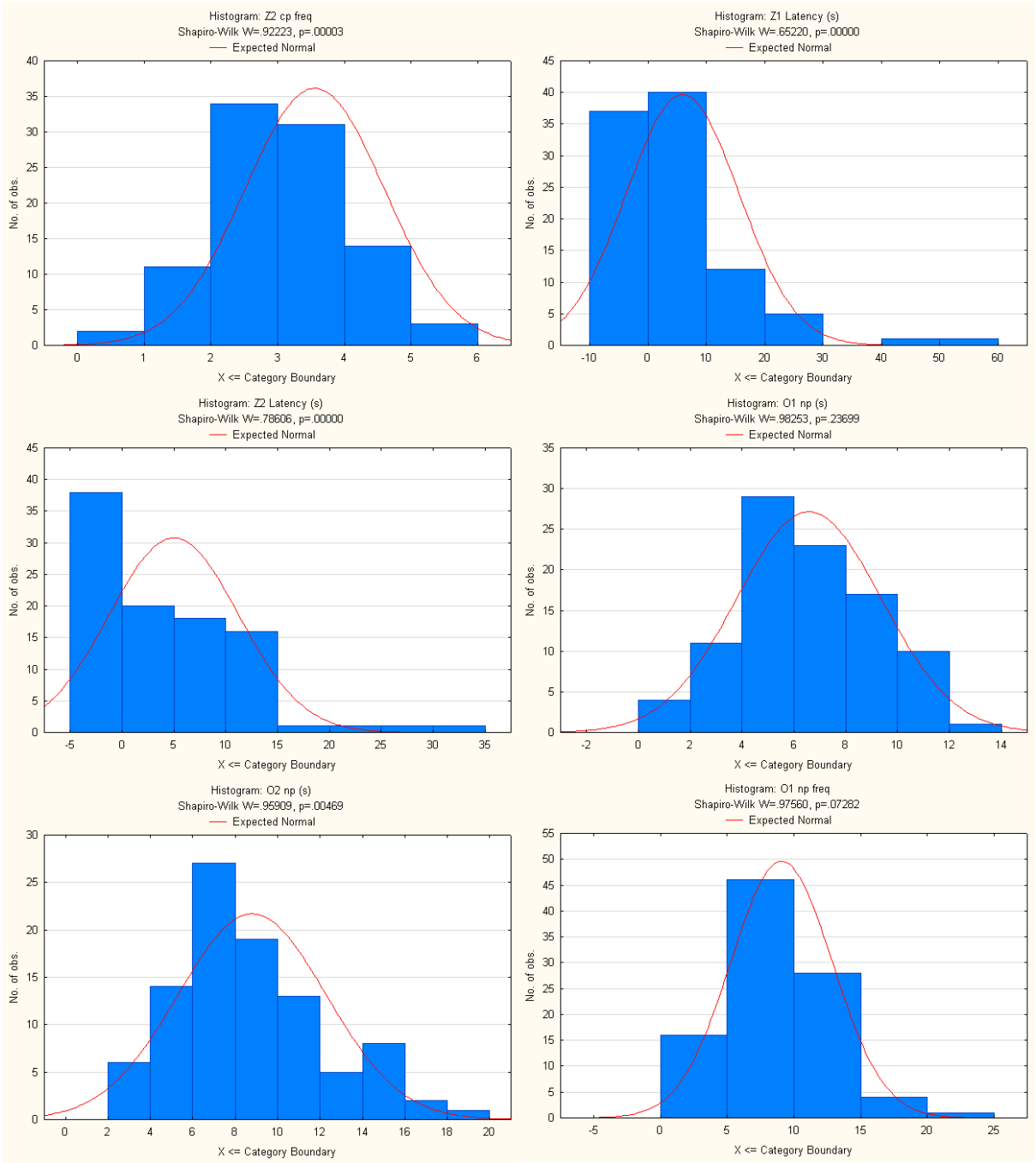


Figure 77- NOR P.2 1 MIN, Histograms 2/3

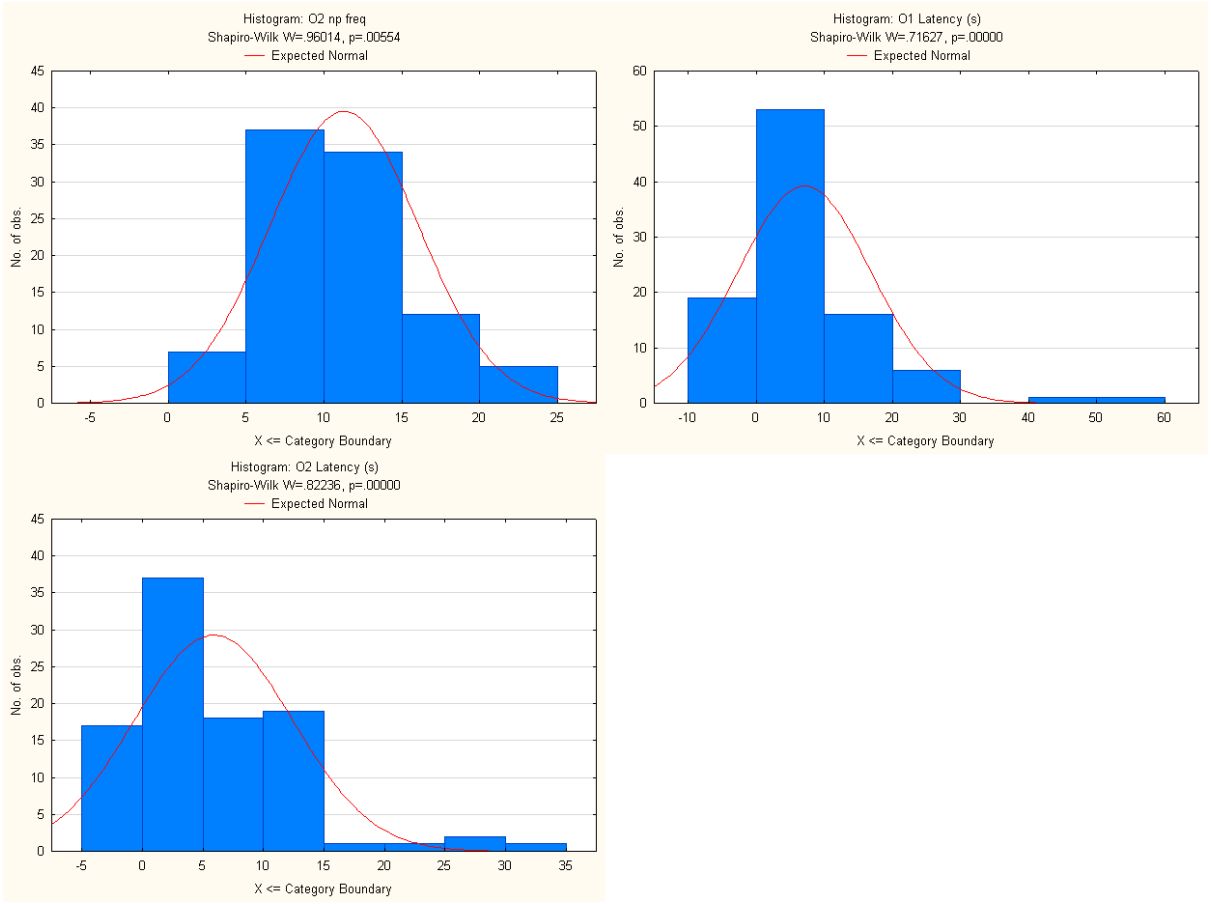


Figure 78- NOR P.2 1 MIN, Histograms 3/3

Table 41- NOR P.2 1 MIN, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
1m Distance (cm)	F	S	24	758.727	206.869	932.213	158.0102
1m Distance (cm)	F	I	22	739.428	466.385	930.645	117.1750
1m Distance (cm)	M	S	25	630.533	303.033	843.794	112.0734
1m Distance (cm)	M	I	24	580.823	419.607	824.254	92.9715

Variable	Sex_Treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
1m Q1 + Q2 (s)	FS	24	34.400	11.200	46.200	32.300	38.300
1m Q3 + Q4 (s)	FS	24	25.600	13.800	48.800	21.700	27.700
1m Q1 (s) Raw	FS	24	17.100	3.400	22.600	14.300	19.100
1m Q1 (s) Corrected	FS	24	18.100	7.200	25.400	16.300	20.500
1m Q2 (s)	FS	24	18.100	7.200	25.400	16.300	20.500
1m Q1 freq	FS	24	4.000	1.000	6.000	3.000	4.500
1m Q2 freq	FS	24	4.000	1.000	6.000	3.000	4.500
1m O1 (s) Raw	FS	24	7.000	1.600	11.600	4.400	8.100
1m O1 (s) Corrected	FS	24	8.100	3.000	14.200	6.800	9.700
1m O2 (s)	FS	24	8.100	3.000	14.200	6.800	9.700
1m O1 freq Raw	FS	24	9.000	2.000	21.000	6.500	12.000
1m O1 freq Corrected	FS	24	11.000	1.000	24.000	9.000	13.000
1m O2 freq	FS	24	11.000	1.000	24.000	9.000	13.000
1m O1 lat (s)	FS	24	2.900	0.000	25.800	0.400	7.200
1m O2 lat (s)	FS	24	4.500	0.000	18.800	0.300	8.300
1m Q1 + Q2 (s)	FI	22	37.500	20.600	48.600	33.800	41.200
1m Q3 + Q4 (s)	FI	22	22.500	11.400	39.400	18.800	26.200
1m Q1 (s) Raw	FI	22	17.100	3.600	27.400	13.800	19.400
1m Q1 (s) Corrected	FI	22	20.200	7.200	28.600	15.800	26.200
1m Q2 (s)	FI	22	20.200	7.200	28.600	15.800	26.200
1m Q1 freq	FI	22	4.000	2.000	5.000	3.000	4.000
1m Q2 freq	FI	22	4.000	2.000	6.000	3.000	5.000
1m O1 (s) Raw	FI	22	6.300	1.000	13.400	4.600	9.000
1m O1 (s) Corrected	FI	22	7.900	3.000	16.600	6.600	12.000
1m O2 (s)	FI	22	7.900	3.000	16.600	6.600	12.000
1m O1 freq Raw	FI	22	9.000	3.000	16.000	6.000	13.000
1m O1 freq Corrected	FI	22	12.500	3.000	23.000	10.000	16.000
1m O2 freq	FI	22	12.500	3.000	23.000	10.000	16.000
1m O1 lat (s)	FI	22	3.000	0.000	19.800	0.200	8.200
1m O2 lat (s)	FI	22	4.000	0.000	26.800	0.400	9.600
1m Q1 + Q2 (s)	MS	25	34.800	18.400	60.000	30.000	38.400
1m Q3 + Q4 (s)	MS	25	25.200	0.000	41.600	21.600	30.000
1m Q1 (s) Raw	MS	25	16.400	6.800	27.600	14.600	20.000
1m Q1 (s) Corrected	MS	25	16.800	7.400	47.600	13.000	21.800
1m Q2 (s)	MS	25	16.800	7.400	47.600	13.000	21.800
1m Q1 freq	MS	25	3.000	2.000	5.000	3.000	4.000
1m Q2 freq	MS	25	3.000	2.000	6.000	3.000	4.000
1m O1 (s) Raw	MS	25	5.400	1.200	12.000	4.400	8.800
1m O1 (s) Corrected	MS	25	7.800	3.200	18.200	5.800	9.400
1m O2 (s)	MS	25	7.800	3.200	18.200	5.800	9.400
1m O1 freq Raw	MS	25	8.000	3.000	19.000	6.000	11.000
1m O1 freq Corrected	MS	25	11.000	4.000	25.000	7.000	13.000
1m O2 freq	MS	25	11.000	4.000	25.000	7.000	13.000
1m O1 lat (s)	MS	25	4.400	0.000	19.200	0.800	10.800
1m O2 lat (s)	MS	25	3.200	0.000	20.200	0.200	8.200
1m Q1 + Q2 (s)	MI	24	35.000	23.000	49.800	31.600	40.300
1m Q3 + Q4 (s)	MI	24	25.000	10.200	37.000	19.700	28.400
1m Q1 (s) Raw	MI	24	15.100	5.400	28.000	12.900	19.600
1m Q1 (s) Corrected	MI	24	21.000	9.400	26.600	17.200	24.400
1m Q2 (s)	MI	24	21.000	9.400	26.600	17.200	24.400
1m Q1 freq	MI	24	3.000	1.000	4.000	2.000	4.000
1m Q2 freq	MI	24	3.000	2.000	5.000	3.000	4.000
1m O1 (s) Raw	MI	24	6.000	1.800	11.400	4.400	8.600
1m O1 (s) Corrected	MI	24	9.100	4.600	17.800	6.900	12.300
1m O2 (s)	MI	24	9.100	4.600	17.800	6.900	12.300
1m O1 freq Raw	MI	24	9.000	1.000	16.000	6.000	11.000
1m O1 freq Corrected	MI	24	9.000	4.000	21.000	7.000	13.000
1m O2 freq	MI	24	9.000	4.000	21.000	7.000	13.000
1m O1 lat (s)	MI	24	5.400	0.000	45.200	0.400	19.200
1m O2 lat (s)	MI	24	7.700	0.000	34.200	0.400	11.900

Table 42- NOR P.2 1 MIN, Distance travelled (cm) statistics

Parametric factorial ANOVA for all sex and housing groups. Followed by Bonferroni post hoc.

Univariate Tests of Significance for Distance (cm) (Spreadsheet33)
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	43495128	1	43495128	2904.288	0.000000
Sex	487318	1	487318	32.540	0.000000
Treatment	28214	1	28214	1.884	0.173262
Sex*Treatment	5479	1	5479	0.366	0.546777
Error	1362832	91	14976		

Bonferroni test; variable Distance (cm) (Spreadsheet33)
Probabilities for Post Hoc Tests
Error: Between MS = 14976., df = 91.000

Cell No.	Sex	{1} 749.50	{2} 606.19
1	F		0.000000
2	M	0.000000	

Table 43- NOR P.2 1 MIN, Quadrants 1+2 vs. Quadrants 3+4 (s) statistics

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

All Groups
Friedman ANOVA and Kendall Coeff. of Concordance (2.2 P2 NOR Raw (1 min))
ANOVA Chi Sqr. (N = 95, df = 1) = 43.57447 p = .00000
Coeff. of Concordance = .45868 Aver. rank r = .45292

Variable	Average Rank	Sum of Ranks	Mean	Std.Dev.
1m Q1 + Q2 (s	1.836842	174.5000	35.42526	7.269946
1m Q3 + Q4 (s	1.163158	110.5000	24.57263	7.269734

Aggregate Results
Wilcoxon Matched Pairs Test (2.2 P2 NOR Raw (1 min))
Marked tests are significant at p <.05000

Pair of Variables	Sex_Treatment	Valid N	T	Z	p-value
Q1 + Q2 (s) & Q3 + Q4 (s)	FS	24	45.00000	3.000000	0.002700
Q1 + Q2 (s) & Q3 + Q4 (s)	FI	22	21.00000	3.425126	0.000615
Q1 + Q2 (s) & Q3 + Q4 (s)	MS	24	57.50000	2.642857	0.008221
Q1 + Q2 (s) & Q3 + Q4 (s)	MI	24	32.00000	3.371429	0.000748

Table 44- NOR P.2 1 MIN, Quadrant 1 vs. Quadrant 2 (s) statistics

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

All Groups
Friedman ANOVA and Kendall Coeff. of Concordance (2.2 P2 NOR Raw (1 min))
ANOVA Chi Sqr. (N = 95, df = 1) = 8.340426 p = .00388
Coeff. of Concordance = .08779 Aver. rank r = .07809

Variable	Average Rank	Sum of Ranks	Mean	Std.Dev.
Q1 (s)	1.352632	128.5000	16.47368	4.982015
Q2 (s)	1.647368	156.5000	18.95158	6.179562

All Groups
Wilcoxon Matched Pairs Test (2.2 P2 NOR Raw (1 min))
Marked tests are significant at p <.05000

Pair of Variables	Valid N	T	Z	p-value
Q1 (s) & Q2 (s)	94	1438.500	2.994122	0.002753

Table 45- NOR P.2 1 MIN, Quadrant 1 vs. Quadrant 2 (number of entries) statistics

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups.

All Groups
Friedman ANOVA and Kendall Coeff. of Concordance (2.2 P2 NOR Raw (1 min))
ANOVA Chi Sqr. (N = 95, df = 1) = .5454545 p = .46018
Coeff. of Concordance = .00574 Aver. rank r = -.0048

Variable	Average Rank	Sum of Ranks	Mean	Std.Dev.
Q1 freq	1.468421	139.5000	3.442105	1.028486
Q2 freq	1.531579	145.5000	3.557895	1.048969

Table 46- NOR P.2 1 MIN, Object 1 vs. Object 2 (s) statistics

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

All Groups
Friedman ANOVA and Kendall Coeff. of Concordance (2.2 P2 NOR Raw (1 min))
ANOVA Chi Sqr. (N = 95, df = 1) = 20.31868 p = .00001
Coeff. of Concordance = .21388 Aver. rank r = .20552

Variable	Average Rank	Sum of Ranks	Mean	Std.Dev.
O1 np (s)	1.273684	121.0000	6.581053	2.792554
O2 np (s)	1.726316	164.0000	8.795789	3.495283

All Groups
Wilcoxon Matched Pairs Test (2.2 P2 NOR Raw (1 min))
Marked tests are significant at p <.05000

Pair of Variables	Valid N	T	Z	p-value
O1 np (s) & O2 np (s)	91	957.5000	4.494202	0.000007

Table 47- NOR P.2 1 MIN, Object 1 vs. Object 2 (number of approaches) statistics

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

Variable	All Groups Friedman ANOVA and Kendall Coeff. of Concordance (2.2 P2 NOR Raw (1 min)) ANOVA Chi Sqr. (N = 95, df = 1) = 13.46154 p = .00024 Coeff. of Concordance = .14170 Aver. rank r = .13257			
	Average Rank	Sum of Ranks	Mean	Std.Dev.
O1 np freq	1.315789	125.0000	9.07368	3.821112
O2 np freq	1.684211	160.0000	11.30526	4.791557

Pair of Variables	All Groups Wilcoxon Matched Pairs Test (2.2 P2 NOR Raw (1 min)) Marked tests are significant at p <.05000			
	Valid N	T	Z	p-value
O1 np freq & O2 np freq	91	1142.000	3.763969	0.000167

Table 48- NOR P.2 1 MIN, Object1 vs. Object 2 (latency of approach, s) statistics

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups.

Variable	All Groups Friedman ANOVA and Kendall Coeff. of Concordance (Spreadsheet51) ANOVA Chi Sqr. (N = 96, df = 1) = .0416667 p = .83826 Coeff. of Concordance = .43E-3 Aver. rank r = -.0101			
	Average Rank	Sum of Ranks	Mean	Std.Dev.
O1 Latency (s)	1.489583	143.0000	7.089583	9.764506
O2 Latency (s)	1.510417	145.0000	5.879167	6.541944

A.2.2.2 P.2- FIVE MINUTES

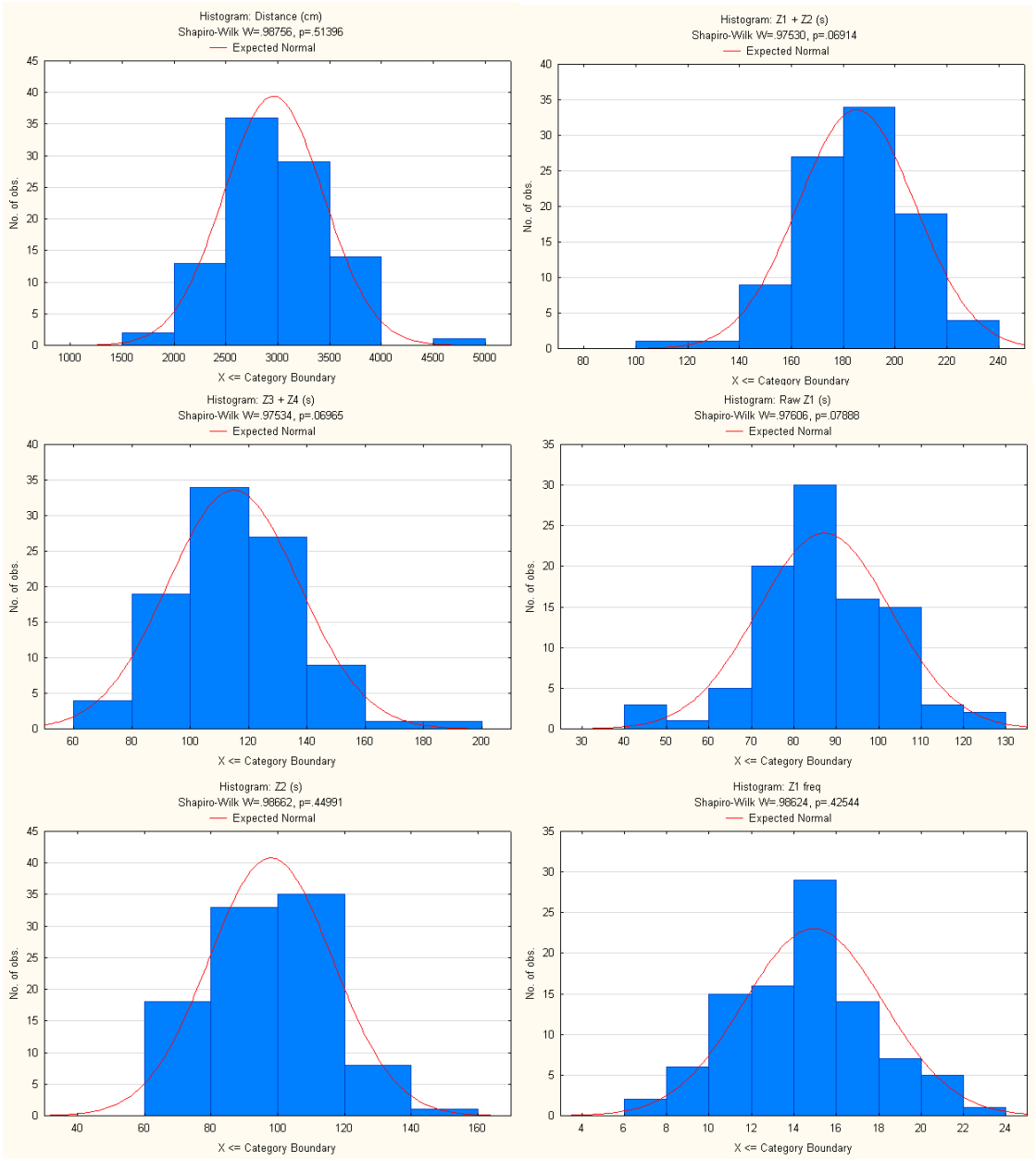


Figure 79- NOR P.2 5 MIN, Histograms 1/2

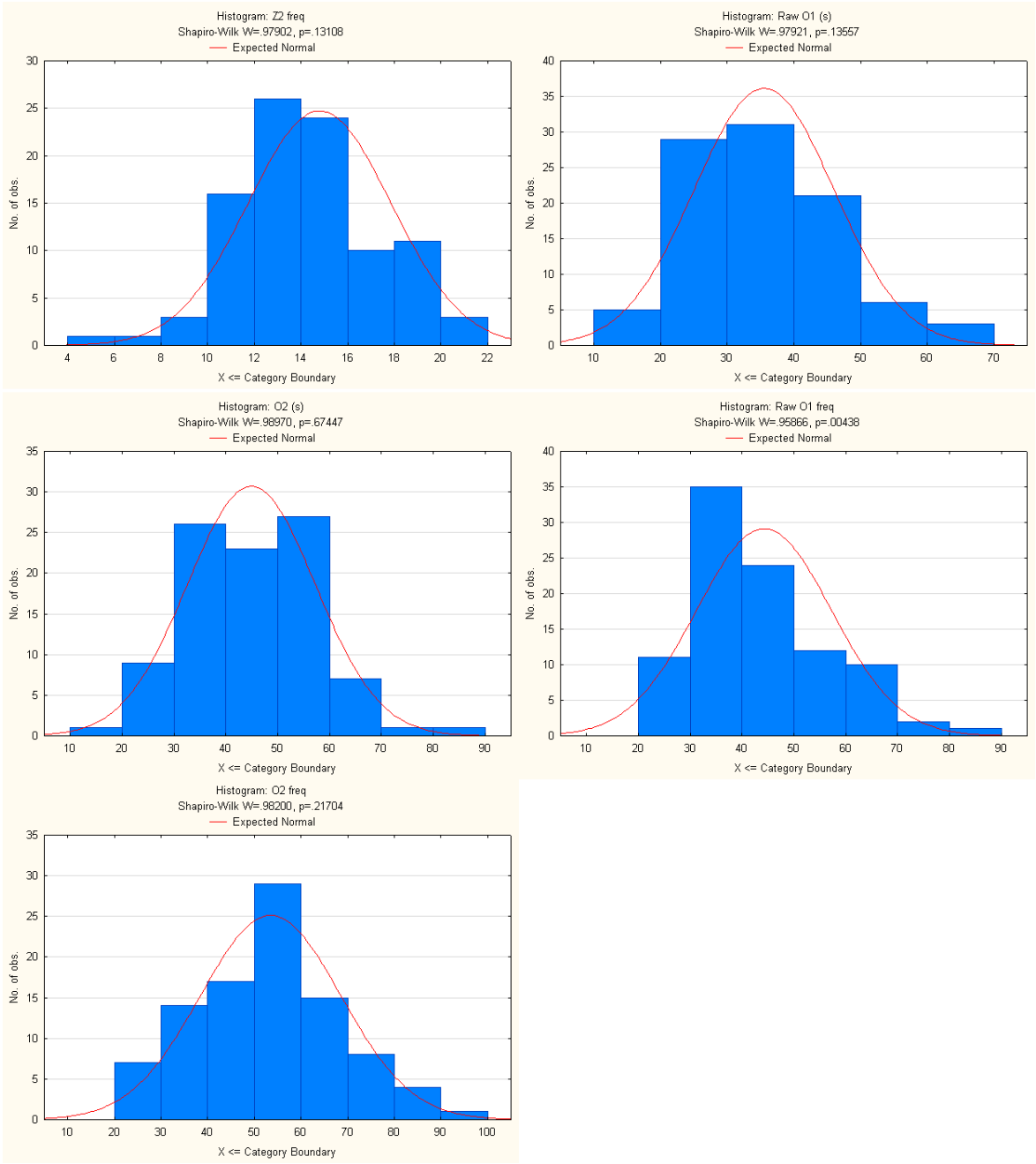


Figure 80- NOR P.2 5 MIN, Histograms 2/2

Table 49- NOR P.2 5 MIN, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
5m Distance (cm)	F	S	24	3183.942	1749.654	3920.671	471.6983
5m Q1 + Q2 (s)	F	S	24	190.050	143.600	218.600	19.8577
5m Q3 + Q4 (s)	F	S	24	109.942	81.400	156.400	19.8667
5m Q1 (s) Raw	F	S	24	88.075	65.200	109.800	12.5996
5m Q1 (s) Corrected	F	S	24	101.975	75.400	145.800	18.6324
5m Q2 (s)	F	S	24	101.975	75.400	145.800	18.6324
5m Q1 freq	F	S	24	16.542	9.000	23.000	3.4133
5m Q2 freq	F	S	24	15.917	6.000	22.000	3.3740
5m O1 (s) Raw	F	S	24	33.917	16.600	63.200	11.3438
5m O1 (s) Corrected	F	S	24	44.108	27.800	61.000	10.0716
5m O2 (s)	F	S	24	44.108	27.800	61.000	10.0716
5m Distance (cm)	F	I	22	3273.660	2489.284	4504.302	471.6664
5m Q1 + Q2 (s)	F	I	22	181.436	146.200	208.400	16.5481
5m Q3 + Q4 (s)	F	I	22	118.564	91.600	153.800	16.5481
5m Q1 (s) Raw	F	I	22	89.600	66.800	119.600	12.8987
5m Q1 (s) Corrected	F	I	22	91.836	62.400	112.600	15.5215
5m Q2 (s)	F	I	22	91.836	62.400	112.600	15.5215
5m Q1 freq	F	I	22	16.000	11.000	22.000	3.0079
5m Q2 freq	F	I	22	16.045	10.000	22.000	2.9355
5m O1 (s) Raw	F	I	22	34.655	20.200	65.600	10.7381
5m O1 (s) Corrected	F	I	22	40.945	16.800	60.800	11.9601
5m O2 (s)	F	I	22	40.945	16.800	60.800	11.9601
5m Distance (cm)	M	S	25	2720.476	1991.127	3732.307	388.8122
5m Q1 + Q2 (s)	M	S	25	183.672	106.800	225.600	27.8913
5m Q3 + Q4 (s)	M	S	25	116.328	74.400	193.200	27.8913
5m Q1 (s) Raw	M	S	25	87.224	42.800	124.600	21.0354
5m Q1 (s) Corrected	M	S	25	96.448	63.600	127.200	17.9470
5m Q2 (s)	M	S	25	96.448	63.600	127.200	17.9470
5m Q1 freq	M	S	25	13.560	7.000	18.000	2.8148
5m Q2 freq	M	S	25	14.160	10.000	21.000	2.8384
5m O1 (s) Raw	M	S	25	37.768	15.800	60.800	11.3740
5m O1 (s) Corrected	M	S	25	47.960	30.400	72.200	12.1843
5m O2 (s)	M	S	25	47.960	30.400	72.200	12.1843
5m Distance (cm)	M	I	24	2695.624	2004.405	3161.576	281.5319
5m Q1 + Q2 (s)	M	I	24	185.050	138.800	222.800	24.1506
5m Q3 + Q4 (s)	M	I	24	114.908	77.200	161.200	24.2043
5m Q1 (s) Raw	M	I	24	84.050	44.200	123.600	14.9177
5m Q1 (s) Corrected	M	I	24	101.000	63.200	135.800	21.0293
5m Q2 (s)	M	I	24	101.000	63.200	135.800	21.0293
5m Q1 freq	M	I	24	13.792	8.000	20.000	2.9924
5m Q2 freq	M	I	24	13.208	7.000	17.000	2.2063
5m O1 (s) Raw	M	I	24	35.650	19.400	50.800	8.4827
5m O1 (s) Corrected	M	I	24	46.283	21.600	81.800	14.4411
5m O2 (s)	M	I	24	46.283	21.600	81.800	14.4411

Variable	Sex_Treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
5m O1 freq Raw	FS	24	42.000	22.000	67.000	34.500	48.500
5m O1 freq Corrected	FS	24	58.000	26.000	96.000	44.000	63.500
5m O2 freq	FS	24	58.000	26.000	96.000	44.000	63.500
5m O1 freq Raw	FI	22	46.500	24.000	76.000	34.000	51.000
5m O1 freq Corrected	FI	22	51.500	28.000	85.000	40.000	63.000
5m O2 freq	FI	22	51.500	28.000	85.000	40.000	63.000
5m O1 freq Raw	MS	25	39.000	23.000	86.000	33.000	56.000
5m O1 freq Corrected	MS	25	55.000	27.000	85.000	40.000	63.000
5m O2 freq	MS	25	55.000	27.000	85.000	40.000	63.000
5m O1 freq Raw	MI	24	42.500	26.000	74.000	36.500	51.500
5m O1 freq Corrected	MI	24	54.500	33.000	80.000	43.000	59.500
5m O2 freq	MI	24	54.500	33.000	80.000	43.000	59.500

Table 50- NOR P.2 5 MIN, Distance travelled (cm) statistics

Parametric factorial ANOVA for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	835277993	1	835277993	4987.382	0.000000
Sex	6426562	1	6426562	38.373	0.000000
Treatment	24927	1	24927	0.149	0.700548
Sex*Treatment	77768	1	77768	0.464	0.497330
Error	15240520	91	167478		

Univariate Tests of Significance for Distance (cm) (Spreadsheet217)
Sigma-restricted parameterization
Effective hypothesis decomposition

Bonferroni test; variable Distance (cm) (Spreadsheet217)
Probabilities for Post Hoc Tests
Error: Between MS = 1675E2, df = 91.000

Cell No.	Sex	{1} 3226.9	{2} 2708.3
1	F		0.000000
2	M	0.000000	

Table 51- NOR P.2 5 MIN, Quadrant 1+2 vs. Quadrant 3+4 (s) statistics

Parametric repeated measures ANOVA (quadrants) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	4265354	1	4265354	1.502505E+09	0.000000
Sex	0	1	0	1.159437E+00	0.284429
Treatment	0	1	0	1.159437E+00	0.284429
Sex*Treatment	0	1	0	2.608733E+00	0.109739
Error	0	91	0		
QUAD	233019	1	233019	2.258753E+02	0.000000
QUAD*Sex	89	1	89	8.671689E-02	0.769064
QUAD*Treatment	618	1	618	5.985721E-01	0.441129
QUAD*Sex*Treatment	1189	1	1189	1.152417E+00	0.285882
Error	93878	91	1032		

Repeated Measures Analysis of Variance (Spreadsheet217)
Sigma-restricted parameterization
Effective hypothesis decomposition

Bonferroni test; variable DV_1 (Spreadsheet217)
Probabilities for Post Hoc Tests
Error: Within MS = 1031.6, df = 91.000

Cell No.	ZONES	{1} 114.87	{2} 185.11
1	Q3 + Q4 (s)		0.00
2	Q1 + Q2 (s)	0.00	

Table 52- NOR P.2 5 MIN, Quadrant 1 vs. Quadrant 2 (s) statistics

Parametric repeated measures ANOVA (quadrants) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (Spreadsheet217) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	1623068	1	1623068	6297.775	0.000000
Sex	23	1	23	0.088	0.767623
Treatment	155	1	155	0.602	0.439914
Sex*Treatment	296	1	296	1.147	0.286906
Error	23453	91	258		
QUAD	5303	1	5303	15.887	0.000136
QUAD*Sex	298	1	298	0.894	0.346866
QUAD*Treatment	46	1	46	0.138	0.711544
QUAD*Sex*Treatment	1114	1	1114	3.336	0.071042
Error	30376	91	334		

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet217) Probabilities for Post Hoc Tests Error: Within MS = 333.80, df = 91.000 ZONE	{1} 87.187	{2} 97.926
1	Q1 (s)		0.000107
2	Q2 (s)	0.000107	

Table 53- NOR P.2 5 MIN, Quadrant 1 vs. Quadrant 2 (number of entries) statistics

Parametric repeated measures ANOVA (quadrants) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (Spreadsheet217) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	42107.03	1	42107.03	2807.184	0.000000
Sex	283.56	1	283.56	18.904	0.000036
Treatment	3.80	1	3.80	0.253	0.615865
Sex*Treatment	0.28	1	0.28	0.019	0.891740
Error	1364.98	91	15.00		
QUAD	0.94	1	0.94	0.360	0.549965
QUAD*Sex	1.05	1	1.05	0.404	0.526643
QUAD*Treatment	0.78	1	0.78	0.299	0.585891
QUAD*Sex*Treatment	10.18	1	10.18	3.905	0.051160
Error	237.21	91	2.61		

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet217) Probabilities for Post Hoc Tests Error: Between MS = 15.000, df = 91.000 Sex	{1} 16.130	{2} 13.684
1	F		0.000035
2	M	0.000035	

Table 54- NOR P.2 5 MIN, Object 1 vs. Object 2 (s) statistics

Parametric repeated measures ANOVA (objects) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (Spreadsheet217) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	305782.9	1	305782.9	2034.470	0.000000
Sex	583.6	1	583.6	3.883	0.051815
Treatment	114.6	1	114.6	0.762	0.384867
Sex*Treatment	5.6	1	5.6	0.037	0.847946
Error	13677.4	91	150.3		
OBJECT	4123.2	1	4123.2	36.930	0.000000
OBJECT*Sex	55.9	1	55.9	0.500	0.481138
OBJECT*Treatment	35.5	1	35.5	0.318	0.574483
OBJECT*Sex*Treatment	55.9	1	55.9	0.500	0.481205
Error	10160.1	91	111.6		

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet217) Probabilities for Post Hoc Tests Error: Within MS = 111.65, df = 91.000 OBJECT	{1} 35.539	{2} 44.939
1	O1 np (s)		0.000000
2	O2 np (s)	0.000000	

Table 55- NOR P.2 5 MIN, Object 1 vs. Object 2 (number of approaches) statistics

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

Variable	Friedman ANOVA and Kendall Coeff. of Concordance (Spreadsheet217) ANOVA Chi Sqr. (N = 95, df = 1) = 18.76596 p = .00001 Coeff. of Concordance = .19754 Aver. rank r = .18900 Average Rank	Sum of Ranks	Mean	Std.Dev.
O1 np freq	1.278947	121.5000	44.24211	13.01122
O2 np freq	1.721053	163.5000	53.50526	15.08548

Pair of Variables	All Groups Wilcoxon Matched Pairs Test (Spreadsheet217) Marked tests are significant at p <.05000 Valid N	T	Z	p-value
O1 np freq & O2 np freq	94	882.5000	5.090762	0.000000

Where an equipment bias was evident variables were corrected, see Equation 2.

A.2.3 PHASE 3- NOVEL OBJECT RECOGNITION ANALYSIS

Variables listed as ‘corrected’ were adjusted using the correction factor calculated in phase 2 (Table 40) (Equation 2). Statistical tests were applied to these ‘corrected’ variables.

Table 56- NOR P.3, Data 1/2

Rat #	Sex	Treatment	Sex_Treatment	1m Distance (cm)	1m (s) Raw	Q1 1m (s) Corrected	Q1 1m freq	Q2 1m freq	Q2 1m Disc Index Q	1m (s) Raw	O1 1m (s) Corrected	O1 1m freq	O2 1m freq	1m Disc Index O	1m lat (s)	O1 1m freq	O1 1m Corrected	O1 1m freq	O2 1m freq	1m lat (s)	1m Disc Index O	5m Distance (cm)	5m Raw	Q1 5m (s) Corrected	Q1 5m freq	Q2 5m freq	5m Disc Index Q	5m (s) Raw	O1 5m (s) Corrected	O1 5m freq	O2 5m freq	5m Disc Index O
2	F	S	FS	898.83	16.20	15.28	5.00	4.00	-0.07	8.00	2.73	6.20	10.00	7.86	8.00	3.20	1.80	-0.39	2803.30	115.40	149.62	12.00	14.00	0.27	46.00	56.43	41.40	39.00	0.15
3	F	S	FS	902.04	10.00	15.54	4.00	7.00	-0.36	7.80	8.67	8.80	7.00	14.00	14.00	0.00	0.80	-0.01	3072.45	81.20	102.78	17.00	22.00	-0.12	32.80	44.03	42.80	35.00	0.01
4	F	S	FS	776.79	14.20	12.85	4.00	4.00	-0.16	6.40	7.43	7.60	11.00	12.38	6.00	2.00	0.00	-0.01	2660.47	68.60	65.90	13.00	14.00	-0.08	23.40	24.20	36.60	35.00	-0.20
6	F	I	FI	825.76	27.20	163.20	6.00	4.00	0.85	11.20	89.60	5.40	16.00	32.00	11.00	0.20	8.00	0.89	3361.02	83.60	118.14	76.40	17.00	15.00	0.21	38.40	46.75	27.40	51.00	68.65	46.00	0.26
7	F	I	FI	487.16	25.40	16.58	3.00	2.00	0.30	12.80	5.74	4.00	10.00	10.00	5.00	0.20	14.00	0.18	2526.10	102.00	65.67	57.00	19.00	14.00	0.07	38.80	19.28	19.00	44.00	36.24	29.00	0.01
8	F	I	FI	682.87	17.60	25.18	3.00	4.00	0.38	8.40	11.09	4.20	13.00	14.44	8.00	10.00	6.40	0.45	2594.25	65.20	82.56	81.40	13.00	18.00	0.01	28.20	31.82	32.40	32.00	41.70	43.00	-0.01
9	M	S	MS	505.98	9.40	6.32	2.00	2.00	0.01	3.80	4.47	4.80	6.00	10.50	2.00	1.00	0.00	-0.04	2460.75	78.40	61.41	63.00	15.00	13.00	-0.01	31.40	39.25	31.40	45.00	36.49	28.00	0.11
10	M	S	MS	367.09	20.40	10.10	3.00	1.00	0.76	14.80	9.33	1.40	5.00	2.78	1.00	0.00	58.60	0.74	2177.18	106.80	97.50	51.80	12.00	11.00	0.31	50.40	39.65	26.40	42.00	42.00	24.00	0.20
11	M	S	MS	636.48	21.60	7.85	4.00	3.00	-0.28	6.20	1.65	7.60	12.00	6.86	8.00	2.20	0.60	-0.64	2215.85	82.60	68.21	109.20	12.00	13.00	-0.23	37.20	31.83	44.60	36.00	43.20	33.00	-0.17
12	M	S	MS	473.43	20.40	27.12	4.00	2.00	0.14	7.20	10.40	11.80	15.00	30.00	7.00	0.00	13.20	-0.06	2135.51	112.20	101.73	112.80	14.00	10.00	-0.05	40.20	37.19	65.20	47.00	45.58	39.00	-0.27
13	M	I	MI	508.61	26.40	20.17	4.00	2.00	-0.05	11.20	16.18	7.00	14.00	12.25	6.00	0.20	18.40	0.40	2122.81	122.60	155.52	92.00	13.00	11.00	0.26	48.80	84.02	45.00	56.00	72.80	44.00	0.30
14	M	I	MI	394.77	13.80		3.00	1.00		5.40			10.00			2.00	32.40		1284.21	38.80			6.00	4.00		16.00			27.00			
15	M	I	MI	505.59	12.60	10.69	2.00	3.00	-0.22	6.60	7.29	10.40	9.00	9.90	8.00	15.80	0.40	-0.18	2303.23	90.60	60.69	98.20	11.00	12.00	-0.24	37.00	23.74	41.20	38.00	29.80	42.00	-0.27
16	M	I	MI	557.61	17.40	17.93	4.00	3.00	0.19	10.20	15.52	6.20	8.00	5.09	8.00	0.40	29.20	0.43	1922.82	49.40	60.18	98.20	9.00	9.00	-0.24	26.20	45.57	45.40	29.00	37.57	55.00	0.00
17	F	S	FS	722.46	13.00	16.01	5.00	3.00	-0.07	3.80	6.06	9.80	7.00	8.75	10.00	0.00	12.20	-0.24	3138.07	81.80	75.08	81.40	15.00	13.00	-0.04	22.20	17.24	37.40	44.00	42.00	41.00	-0.37
18	F	S	FS	964.84	15.00	14.46	6.00	5.00	0.04	6.00	12.55	8.40	9.00	12.60	11.00	7.20	5.80	0.20	3633.54	57.60	42.83	78.40	18.00	18.00	-0.29	24.60	25.34	35.60	36.00	27.69	55.00	-0.17
19	F	S	FS	636.13	28.60	18.72	3.00	3.00	0.16	16.20	19.15	8.60	26.00	6.50	8.00	0.40	0.00	0.38	2667.54	94.40	105.89	75.20	11.00	12.00	0.17	43.80	86.38	36.80	61.00	94.27	36.00	0.40
20	F	S	FS	763.54	27.00	61.94	5.00	4.00	0.65	12.00	24.00	5.00	20.00	70.00	9.00	0.80	5.20	0.66	2697.31	92.00	110.63	60.20	12.00	12.00	0.30	43.20	48.82	25.00	50.00	59.09	40.00	0.32
21	F	I	FI	691.04	22.40	50.84	5.00	3.00	0.49	8.00	22.46	12.40	20.00	48.00	12.00	2.20	3.80	0.29	2759.56	66.60	64.38	63.40	13.00	13.00	0.01	26.80	37.88	29.80	47.00	53.71	40.00	0.12
22	F	I	FI	497.72	11.60		2.00	0.00		6.00			9.00			2.20	60.00		1967.32	82.00			13.00	5.00		32.20			48.00			
23	F	I	FI	890.83	26.40	16.74	5.00	4.00	0.19	11.80	4.76	4.20	17.00	28.33	8.00	0.40	8.60	0.06	3051.27	119.00	86.98	72.00	17.00	19.00	0.09	59.20	39.24	29.40	80.00	89.41	44.00	0.14
24	F	I	FI	608.61	11.20	6.02	3.00	3.00	-0.43	6.80	5.70	8.80	4.00	1.50	10.00	0.60	33.20	-0.21	2705.15	95.60	81.32	95.00	14.00	11.00	-0.08	50.20	58.63	49.60	48.00	39.53	51.00	0.08
25	M	S	MS	646.39	12.00	10.55	4.00	3.00	-0.30	4.80	4.80	7.80	8.00	5.00	6.00	0.40	7.40	-0.24	2416.96	111.20	99.25	63.20	12.00	10.00	0.22	51.40	39.57	26.20	42.00	33.35	22.00	0.20
26	M	S	MS	561.09	21.60	42.86	3.00	3.00	0.44	12.60	24.73	9.60	10.00	23.33	8.00	4.20	0.20	0.44	2522.22	67.20	72.02	97.60	10.00	13.00	-0.15	30.20	32.13	45.80	28.00	33.76	41.00	-0.18
27	M	S	MS	582.21	25.80	41.21	4.00	4.00	0.47	11.40	22.11	8.20	14.00	30.33	7.00	0.40	12.60	0.46	1899.85	177.20	197.81	79.80	10.00	8.00	0.43	49.40	77.22	30.00	88.00	121.85	28.00	0.44
28	M	S	MS	622.97	15.80	60.65	3.00	3.00	0.72	6.00	49.64	3.40	8.00	40.00	6.00	4.00	0.60	0.87	2980.94	88.80	89.29	86.40	16.00	16.00	0.02	26.00	29.14	35.40	35.00	46.38	25.00	-0.10
29	M	I	MI	480.90	13.40	15.36	5.00	4.00	-0.23	8.60	9.51	6.00	6.00	4.50	7.00	0.00	22.00	0.23	2442.20	81.00	143.31	109.80	15.00	14.00	0.13	33.80	69.48	38.80	40.00	46.49	39.00	0.28
30	M	I	MI	469.71	19.20	62.58	3.00	2.00	0.66	6.40	29.54	6.20	14.00	31.50	6.00	0.20	1.80	0.65	2210.97	82.60	87.43	83.00	13.00	12.00	0.03	25.40	30.15	46.80	48.00	72.00	42.00	-0.22
31	M	I	MI	612.33	9.40	3.16	3.00	3.00	-0.38	4.80	2.76	5.80	8.00	3.56	4.00	0.00	21.80	-0.36														
32	M	I	MI	589.20	23.00	26.15	4.00	5.00	0.25	7.00	14.82	6.40	13.00	17.33	12.00	0.00	2.20	0.40														
33	F	S	FS	939.81	17.40	18.56	7.00	4.00	-0.05	5.80	7.83	11.20	14.00	23.33	15.00	9.20	6.60	-0.18	2415.37	106.80	94.80	53.40	14.00	11.00	0.28	54.80	64.55	27.40	42.00	33.80	26.00	0.40
34	F	S	FS	746.49	20.60	31.05	4.00	5.00	0.17	6.00	7.24	9.80	10.00	11.43	13.00	1.80	0.20	-0.15	2579.65	119.00	156.07	59.40	16.00	16.00	0.45	36.80	49.30	26.20	64.00	100.80	35.00	0.31
35	F	S	FS	858.48	20.20	35.63	5.00	5.00	0.34	9.60	16.66	7.80	16.00	22.15	13.00	3.60	0.40	0.36	3115.26	91.00	100.14	91.20	17.00	17.00	0.05	34.80	50.04	46.00	57.00	88.53	59.00	0.04
36	F	S	FS	960.90	22.80	20.18	6.00	5.00	0.07	11.20	11.20	6.80	15.00	10.71	13.00	0.00	5.20	0.24	3137.51	110.20	107.22	82.40	18.00	15.00	0.13	41.80	43.16	33.00	54.00	51.58	60.00	0.13
37	F	I	FI	642.01	14.20	7.28	4.00	4.00	-0.53	5.80	1.58	10.20	7.00	3.73	11.00	4.00	0.40	-0.73	2856.68	81.60	76.83	92.60	17.00	15.00	-0.09	26.00	33.80	40.00	39.00	53.41	54.00	-0.08
38	F	I	FI	831.68	21.00	13.34	4.00	4.00	0.20	8.60	5.28	3.80	18.00	19.38	8.00	3.40	5.40	0.16	2914.43	103.00	68.23	67.00	16.00	13.00	0.01	37.40	36.88	37.60	61.00	62.53	52.00	-0.01
39	F	I	FI	817.44	20.60	21.02	6.00	5.00	0.14	5.80	5.52	4.60	14.00	21.64	15.00	0.20	7.40	0.09	3223.57	96.00	73.81	99.40	18.00	19.00	-0.15	25.20	17.35	31.60	56.00	51.52	68.00	-0.29
40	F	I	FI	918.02	19.40	19.40	6.00	5.00	0.03	10.20	16.85	9.40	18.00	16.71	17.00	0.00	9.20	0.28	3320.25	108.00	120.66	82.80	20.00	17.00	0.19	45.20	88.87	42.40	70.00	84.24	68.00	0.35
41	M	S	MS	634.43	24.40	9.55	4.00	4.00	-0.26	5.20	2.48	7.40	14.00	2.95	16.00	0.00	9.00	-0.50	2297.22	83.60	54.33	93.60	12.00	12.00	-0.27	22.80	16.19	34.80	38.00	32.74	72.00	-0.37
42	M	S	MS	600.13	9.80	12.08	5.00	4.00	-0.40	4.80	8.29	13.60	10.00	15.45	17.00	4.80	1.00	-0.24	2638.13	93.00	108.12	124.40	15.00	13.00	-0.07	30.00	43.92	56.40	48.00	66.89	70.00	-0.12
43	M	S	MS	635.29	17.60	16.67	3.00	3.00	0.02	9.60	13.29	4.60	10.00	10.83	14.00	8.40	6.40	0.49	2529.43	102.20	80.05	120.20	12.00	15.00	-0.20	46.00	40.10	56.40	67.00	53.76	68.00	-0.17
44	M	S	MS	713.31	28.60	9.88	4.00	4.00	-0.24	5.80	2.26	8.20	15.00	7.50	14.00	1.00	0.20	-0.57	3085.83	107.20	77.66	98.80	17.00	16.00	-0.12	35.20	23.00	40.40	58.00	44.90	68.00	-0.27
45	M	I	MI	351.57	20.00	27.88	2.00	4.00	-0.05	7.00	18.20	11.80	11.00	13.44	20.00	2.20	4.40	0.21	2713.84	84.40	90.37	131.60	15.00	18.00	-0.19	28.80	27.34	65.40	45.00	48.38	85.00	-0.41
46	M	I	MI	750.33	24.20	30.45	6.00	5.00	0.17	7.20	6.61	6.20	20.00	16.36	12.00	0.80	0.40	0.03	2971.60	102.60	136.33	98.60	16.00	17.00	0.16	30.60	37.90	27.00	73.00	114.94	55.00	0.17
47	M	I	MI	672.20	20.40	21.68	3.00	4.00	0.14	5.40	5.40	6.40	15.00	28.64	14.00	8.00	0.00	-0.08	2460.21	121.00	76.32	96.00	12.00	15.00	-0.11	37.40	23.16	42.60	82.00	62.05	78.00	-0.30
49	F	S	FS	740.38	15.40	23.80	4.00	5.00	-0.02	5.40	10.09	10.00	10.00	16.67	16.00	2.40	1.20	0.00	3279.17	103.40	122.45	100.20	18.00	20.00	0.10	29.60	42.94	42.40	57.00	83.60	58.00	0.01

Table 57- NOR P.3, Data 2/2

Rat #	Sex	Treatment	Sex_Treatment	1m Distance (cm)	1m (s) Raw	Q1 1m (s) Corrected	Q1 1m freq	Q2 1m (s)	Q2 1m freq	1m Disc Index Q	O1 1m (s) Raw	O1 1m (s) Corrected	O1 1m freq	O2 1m (s)	O2 1m freq	1m Disc Index O	1m Distance (cm)	5m (s) Raw	Q1 5m (s) Corrected	Q1 5m freq	Q2 5m freq	5m Disc Index Q	5m (s) Raw	O1 5m (s) Corrected	O1 5m freq	O2 5m freq	5m Disc Index O
50	F	S	FS	934.31	24.60	10.75	7.00	13.40	6.00	-0.11	8.80	3.34	4.00	18.00	8.00	-0.09	3327.56	86.40	77.43	82.00	19.00	-0.03	34.80	26.10	28.20	60.00	50.00	-0.04
51	F	S	FS	614.23	20.20	21.12	3.00	24.80	4.00	-0.08	11.20	13.21	15.60	14.00	19.00	-0.08	2373.02	83.20	68.47	160.00	13.00	-0.40	36.00	45.19	82.60	50.00	52.63	-0.29
52	F	S	FS	665.87	24.60	27.14	10.40	5.00	2.00	0.45	9.00	7.12	4.40	22.00	24.00	0.24	2423.15	113.80	155.16	93.20	14.00	0.25	31.60	49.63	44.80	61.00	110.56	0.05
53	F	I	FI	868.00	19.00	30.40	11.80	5.00	5.00	0.44	12.00	26.92	5.00	13.00	41.17	0.69	3555.64	95.60	126.12	89.40	19.00	0.17	32.20	54.69	49.00	43.00	57.62	0.19
54	F	I	FI	875.85	18.40	38.78	22.60	5.00	5.00	0.26	8.40	27.20	13.60	14.00	22.40	0.33	2648.03	95.60	108.11	74.80	11.00	0.18	33.40	48.48	33.20	63.00	78.21	55.00	0.09
56	F	I	FI	852.13	23.40	27.37	15.20	6.00	6.00	0.29	14.20	23.98	4.20	12.00	17.25	7.00	1.60	10.20	0.70	3431.32	105.80	71.18	81.80	22.00	19.00	-0.07	41.20	32.96	27.60	46.00	33.29	35.00	0.09
57	M	S	MS	536.53	25.60	37.08	20.40	4.00	3.00	0.29	9.40	25.69	9.20	15.00	22.50	15.00	0.40	2.20	0.47	2341.31	102.60	247.39	77.40	11.00	12.00	0.52	35.00	134.41	39.60	54.00	93.71	47.00	0.54
58	M	S	MS	663.87	15.40	19.83	23.40	4.00	4.00	-0.08	6.60	11.35	8.60	11.00	20.63	18.00	4.00	6.40	0.14	2696.99	69.00	111.68	77.80	12.00	12.00	0.18	30.80	57.62	34.60	37.00	93.19	45.00	0.25
59	M	S	MS	669.17	23.60	25.89	11.80	5.00	3.00	0.37	9.80	8.82	2.40	9.00	6.60	3.00	0.00	8.60	0.57	2948.82	95.40	126.66	96.80	18.00	15.00	0.13	37.80	56.48	28.60	41.00	58.70	33.00	0.33
60	M	S	MS	457.17	28.80	25.08	19.80	4.00	3.00	0.12	13.00	13.34	9.00	14.00	15.27	16.00	0.00	11.20	0.19	2375.63	95.60	95.42	82.80	11.00	11.00	0.07	34.40	41.68	40.00	44.00	42.48	51.00	0.02
61	M	I	MI	479.24	8.20	34.99	6.00	2.00	2.00	0.71	3.20	14.20	4.60	5.00	25.00	6.00	25.80	33.60	0.51	2098.00	70.80	151.53	75.00	8.00	10.00	0.34	32.00	83.46	39.40	35.00	98.27	41.00	0.36
62	M	I	MI	601.12	24.00	46.84	11.40	4.00	3.00	0.61	10.80	40.63	4.80	11.00	11.00	6.00	0.00	4.00	0.79	2685.25	101.80	135.82	86.00	13.00	14.00	0.22	43.40	77.71	36.60	44.00	46.38	33.00	0.36
63	M	I	MI	215.22	21.00	16.30	13.40	1.00	2.00	0.10	14.80	12.11	5.00	8.00	6.86	4.00	30.80	1.80	0.42	1133.95	146.00	122.54	58.00	4.00	4.00	0.36	67.20	59.49	19.20	67.00	61.42	12.00	0.51
64	M	I	MI	525.16	22.40	15.08	18.80	4.00	4.00	-0.11	10.40	5.32	9.00	11.00	9.00	0.00	3.40	2.20	-0.26	2276.53	95.00	108.77	82.40	15.00	13.00	0.14	36.60	46.88	30.40	49.00	85.75	38.00	0.21
65	F	S	FS	700.93	23.40	25.50	16.00	4.00	4.00	0.23	9.40	15.22	5.60	11.00	7.62	10.00	0.00	12.20	0.46	2924.22	77.20	78.67	109.60	18.00	17.00	-0.16	27.00	49.87	49.60	43.00	44.08	55.00	0.00
66	F	S	FS	818.41	15.20	17.40	12.00	5.00	4.00	0.18	8.00	9.40	5.80	11.00	13.44	4.00	2.40	7.40	0.24	2962.31	75.20	77.77	79.80	16.00	14.00	-0.01	25.80	25.66	42.40	41.00	33.83	28.00	-0.25
67	F	S	FS	806.51	15.60	9.36	22.00	5.00	6.00	-0.40	5.00	3.87	7.40	6.00	4.50	12.00	1.20	0.00	-0.31	2964.41	83.60	87.60	62.20	13.00	16.00	0.17	31.00	28.63	22.80	44.00	44.88	27.00	0.11
68	F	S	FS	759.85	16.80	14.52	10.60	5.00	4.00	0.16	5.60	5.44	3.80	11.00	8.00	5.00	0.00	10.20	0.18	2729.77	76.60	72.58	94.00	15.00	14.00	-0.13	33.20	30.36	56.20	39.00	33.75	41.00	-0.30
69	F	I	FI	853.83	21.60	40.20	23.00	5.00	6.00	0.27	11.20	28.80	10.20	10.00	17.27	14.00	0.00	1.00	0.48	3385.54	118.20	147.75	92.20	17.00	20.00	0.23	55.20	84.97	45.20	40.00	50.75	39.00	0.31
70	F	I	FI	650.21	23.20	32.89	16.80	5.00	4.00	0.32	10.40	13.87	6.80	9.00	16.50	5.00	2.60	0.00	0.34	2550.27	86.00	99.61	124.40	14.00	14.00	-0.11	45.00	47.03	70.00	30.00	38.48	33.00	-0.20
71	F	I	FI	821.91	11.60	12.75	16.40	5.00	6.00	-0.13	7.40	9.99	3.00	5.00	6.67	4.00	2.40	1.20	0.54	2920.97	77.00	102.89	98.60	15.00	19.00	0.02	37.40	56.00	23.80	26.00	36.83	32.00	0.40
72	F	I	FI	859.89	14.20	12.89	18.00	6.00	5.00	-0.17	8.80	13.66	2.60	7.00	7.00	5.00	0.00	11.20	0.68	2894.98	61.00	74.35	99.20	15.00	14.00	-0.14	25.00	41.11	15.20	25.00	30.73	32.00	0.46
73	M	S	MS	611.77	26.60	34.51	16.20	5.00	4.00	0.36	10.60	31.21	7.20	19.00	16.63	15.00	0.00	1.40	0.63	2484.81	98.60	142.16	104.20	15.00	15.00	0.15	39.60	58.45	47.00	60.00	64.86	53.00	0.11
74	M	S	MS	882.09	19.20	15.17	17.40	7.00	6.00	-0.07	10.20	9.63	4.00	16.00	9.60	8.00	0.60	1.20	0.41	3104.68	90.80	94.09	101.20	24.00	21.00	-0.04	33.60	47.82	25.20	50.00	48.78	47.00	0.31
75	M	S	MS	627.45	11.60	13.46	18.40	4.00	4.00	-0.15	6.00	4.14	6.60	7.00	9.80	12.00	16.40	0.00	-0.23	2567.38	60.00	74.51	93.40	14.00	13.00	-0.11	29.20	35.20	33.00	30.00	71.74	47.00	0.03
76	M	S	MS	667.57	19.80	23.64	20.40	6.00	4.00	0.07	10.80	17.28	9.60	12.00	19.71	9.00	0.60	7.80	0.29	2441.51	74.00	75.70	145.40	18.00	17.00	-0.32	31.60	45.37	47.60	33.00	51.48	69.00	-0.02
77	M	I	MI	579.12	15.00	27.68	23.40	2.00	4.00	0.08	9.00	23.56	10.00	10.00	11.11	14.00	1.20	0.00	0.40	2552.34	98.60	125.89	91.40	16.00	17.00	0.16	52.40	91.20	26.20	49.00	62.00	44.00	0.55
78	M	I	MI	379.30	26.00	32.59	20.40	3.00	3.00	0.23	13.40	33.75	7.40	12.00	16.80	13.00	0.20	1.60	0.64	2390.11	105.00	132.55	105.20	14.00	13.00	0.12	58.60	92.73	36.00	50.00	74.32	50.00	0.44
79	M	I	MI	325.06	20.20	18.65	27.60	2.00	2.00	-0.19	8.40	11.42	3.40	7.00	5.25	7.00	15.40	0.00	0.54	1608.86	92.60	105.63	99.20	8.00	9.00	0.03	38.20	38.72	15.60	32.00	36.77	27.00	0.43
80	M	I	MI	390.76	16.00	18.24	33.00	3.00	3.00	-0.29	5.20	4.51	5.20	5.00	2.19	10.00	0.00	5.60	-0.07	1985.42	84.20	94.46	153.20	12.00	14.00	-0.24	40.00	49.96	48.40	38.00	31.85	55.00	0.02
81	F	S	FS	967.45	21.60	24.08	20.20	6.00	5.00	0.09	11.20	15.35	4.60	15.00	18.33	13.00	0.20	0.80	0.54	3425.42	80.80	105.12	144.20	17.00	19.00	-0.16	41.00	52.99	24.20	58.00	77.33	58.00	0.37
82	F	S	FS	887.41	20.00	34.14	21.80	4.00	6.00	0.22	10.80	32.40	5.00	17.00	37.40	9.00	2.80	5.80	0.73	2844.54	87.40	175.04	123.40	12.00	15.00	0.17	53.20	187.80	33.60	49.00	138.55	55.00	0.70
83	F	S	FS	905.67	19.80	22.16	18.80	4.00	5.00	0.08	9.60	14.16	6.40	14.00	28.00	10.00	2.00	3.00	0.38	3174.76	102.80	180.84	111.40	14.00	19.00	0.24	51.40	117.08	24.80	71.00	148.17	42.00	0.65
84	F	S	FS	890.16	13.60	13.14	31.60	3.00	7.00	-0.41	4.40	8.00	6.40	9.00	16.71	11.00	12.40	11.20	0.11	3010.97	71.20	135.82	151.60	13.00	20.00	-0.05	31.00	85.95	26.20	59.00	110.63	47.00	0.53
85	F	I	FI	686.89	13.20	23.37	24.40	4.00	4.00	-0.02	6.40	12.80	6.40	10.00	21.67	11.00	5.20	0.20	0.33	2395.65	89.60	103.37	88.20	12.00	12.00	0.08	34.60	36.31	18.40	58.00	59.23	38.00	0.33
86	F	I	FI	999.76	25.20	48.21	18.80	7.00	6.00	0.44	13.60	24.11	8.20	19.00	38.00	11.00	0.00	2.20	0.49	3518.58	75.00	105.56	111.40	19.00	19.00	-0.03	33.80	55.08	42.20	59.00	86.23	51.00	0.13
87	F	I	FI	555.49	19.00	28.38	11.60	4.00	3.00	0.42	8.00	22.32	2.80	9.00	20.57	5.00	0.00	1.00	0.78	2250.00	76.80	89.99	114.20	14.00	14.00	-0.12	44.00	76.24	18.80	41.00	47.56	37.00	0.60
88	F	I	FI	734.35	20.40	20.61	26.20	4.00	6.00	-0.12	10.60	12.92	5.20	9.00	7.71	10.00	7.20	1.40	0.43	2503.99	85.20	98.04	115.80	12.00	14.00	-0.08	44.80	45.04	38.00	34.00	35.10	54.00	0.08
89	M	S	MS	680.70	25.00	47.79	14.60	4.00	4.00	0.53	11.20	44.80	3.00	18.00	54.00	8.00	1.20	0.00	0.87	2301.43	135.40	202.47	71.40	12.00	12.00	0.48	63.80	122.75	23.60	66.00	109.24	51.00	0.68
90	M	S	MS	603.94	14.60	19.47	24.00	4.00	6.00	-0.10	9.00	16.88	4.40	13.00	14.30	12.00	6.80	0.00	0.59	1945.82	112.20	149.41	107.20	10.00	13.00	0.16	63.60	107.80	29.60	47.00	47.84	51.00	0.57
91	M	S	MS	456.38	24.20	21.24	25.60	2.00	3.00	-0.09	14.40	30.76	8.20	24.00	26.40	11.00	8.20	15.60	0.58	1905.53	94.00	147.43	108.40	11.00	13.00	0.15	42.20	82.07	44.80	63.00	115.98	48.00	0.29
92	M	I	MI	373.84	10.80	19.20	23.80	2.00	2.00	-0.11	5.40	5.98	7.40	7.00	21.00	11.00	0.00	19.40	-0.11	1650.67	51.00	80.74	120.60	7.00	11.00	-0.20	30.20	48.67	38.00	18.00	21.79	55.00	0.12
93	M	I	MI	589.20	16.80	45.04	22.20	3.00	4.00	0.34	7.60	11.94	9.20	8.00	18.67	12.00	0.00	3.20	0.13	2201.54	82.80	139.38	120.20	12.00	11.00	0.07	43.20	60.14	52.40	37.00	44.85	49.00	0.07
94	M	I	MI	383.65	13.00	59.43	17.40	1.00	4.00	0.55	3.60	14.00	5.80	8.00	96.00	5.00	37.40	13.20	0.41	1618.36	65.60	114.21	45.80	7.00	9.00	0.43	25.80	26.42	18.00	28.00	47.68	16.00	0.19
95	M	S	MS	533.77	18.60	25.10	11.40	3.00	2.00	0.38	1.40	2.30	1.40	5.00	9.29	4.00	5.80	7.00	0.24	1616.75	87.80	197.32	122.20	6.00	8.00	0.24	33.40	89.92	20.00	32.00	76.80	33.00	0.64
96	M	S	MS	65.40	40.40	27.04	11.20	4.00	6.00	0.41		0.00	0.00	0.00	0.00	0.00	60.00	60.00	0.00	844.16	83.80	87.51	43.40	8.00	8.00	0.34	10.00	11.96	9.80	7.00	6.78	21.00	0.10
97	M	I	MI	374.04	30.20	47.24	15.40	2.00	3.00	0.51	27.00	47.25	1.80	14.00	15.17	4.00	1.60	0.00	0.93	1239.92	138.20	121.00	111.20	6.00	8.00	0.04	121.80	102.94	50.80	49.00	36.35	40.00	0.34
98	M	I	MI	437.14	25.80	31.48	13.20	3.00	3.00	0.41	14.20	13.61	7.00	13.00	16.90	12.00	3.40	0.00	0.32	1202.67	210.20	276.98	42.80	5.00	7.00	0.73	119.20	147.50	19.60	82.00	83.71	25.00	0.77
99	F	S	FS	677.06	20.80	24.86	12.60	3.00	5.00	0.33	11.80	15.54	5.40	5.00	8.13	8.00	4.20	3.00	0.48	1903.04	81.20	85.15	144.60	9.00	10.00	-0.26	48.20	49.64	110.60	25.00	39.06	52.00	-0.38
100	F	I	FI	466.45	28.20	23.61	16.40	3.00	3.00	0.18	13.20	13.20	9.20	8.00	9.33	9.00	0.20	1.40	0.18	1352.64	238.00	190.79	29.40	6.00	6.00	0.73	147.80	167.86	15.60	102.00	84.00	14.00	0.83

A.2.3.1 P.3- FIRST MINUTE

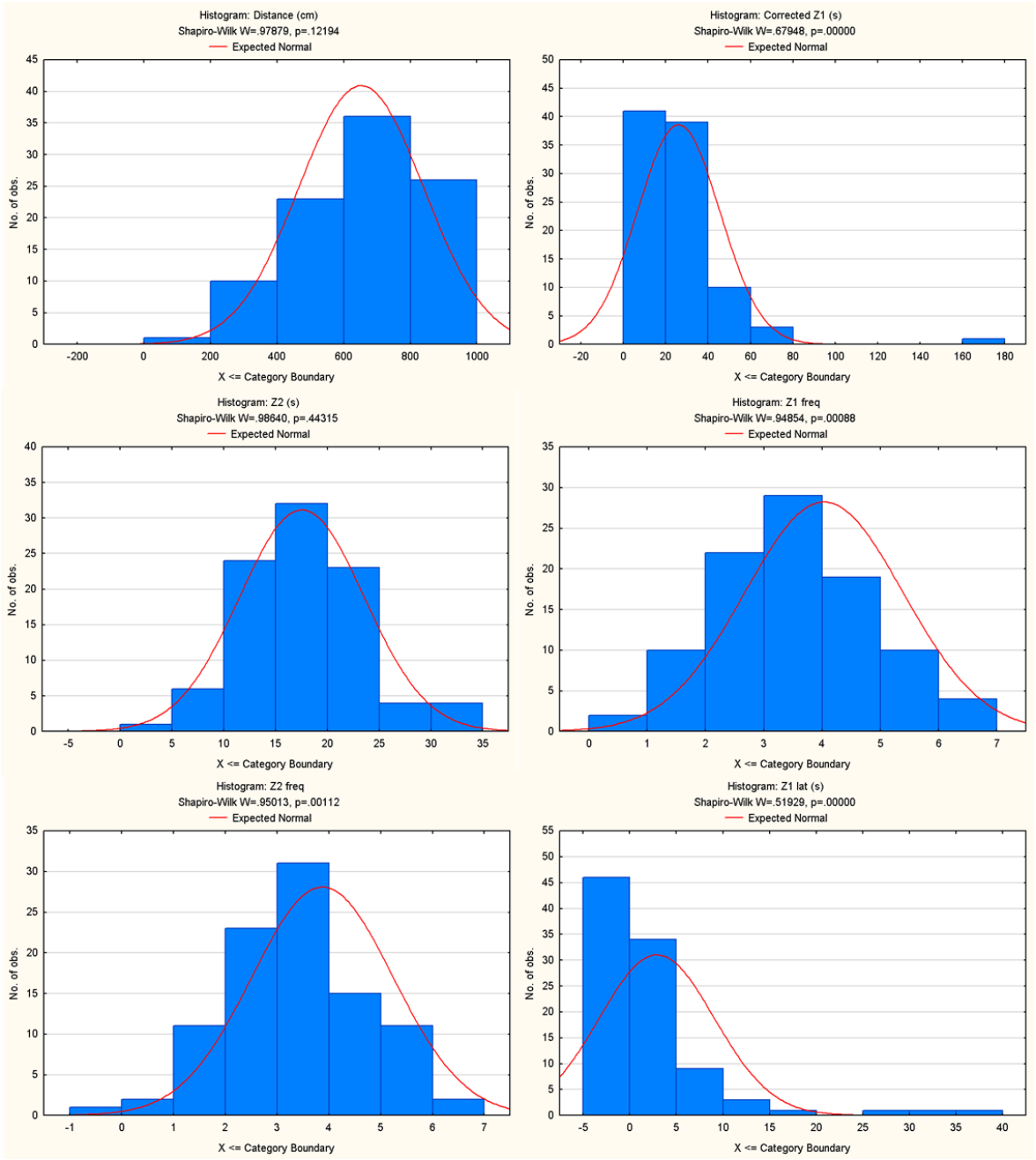


Figure 81- NOR P.3 1 MIN, Histograms 1/3

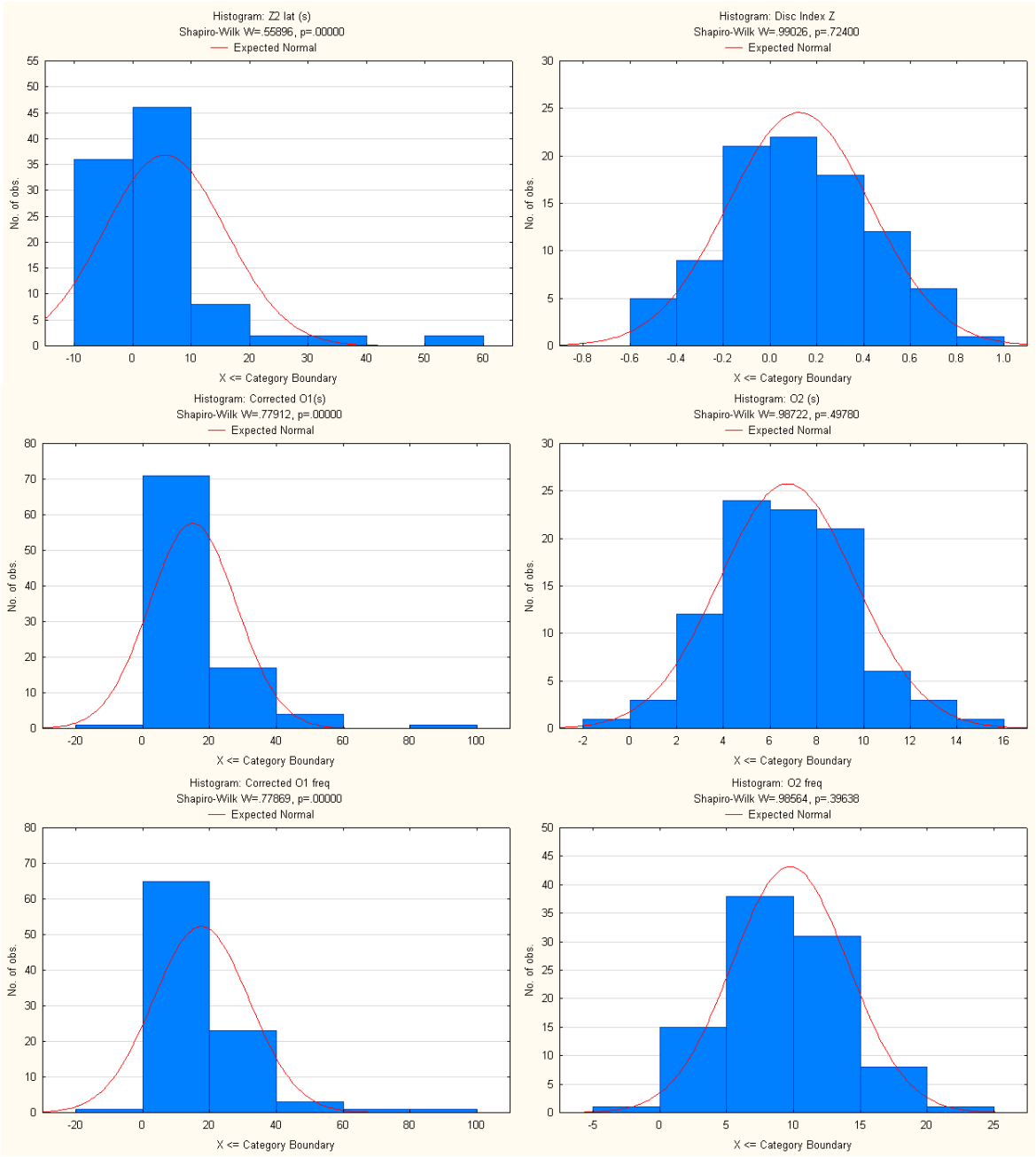


Figure 82- NOR P.3 1 MIN, Histograms 2/3

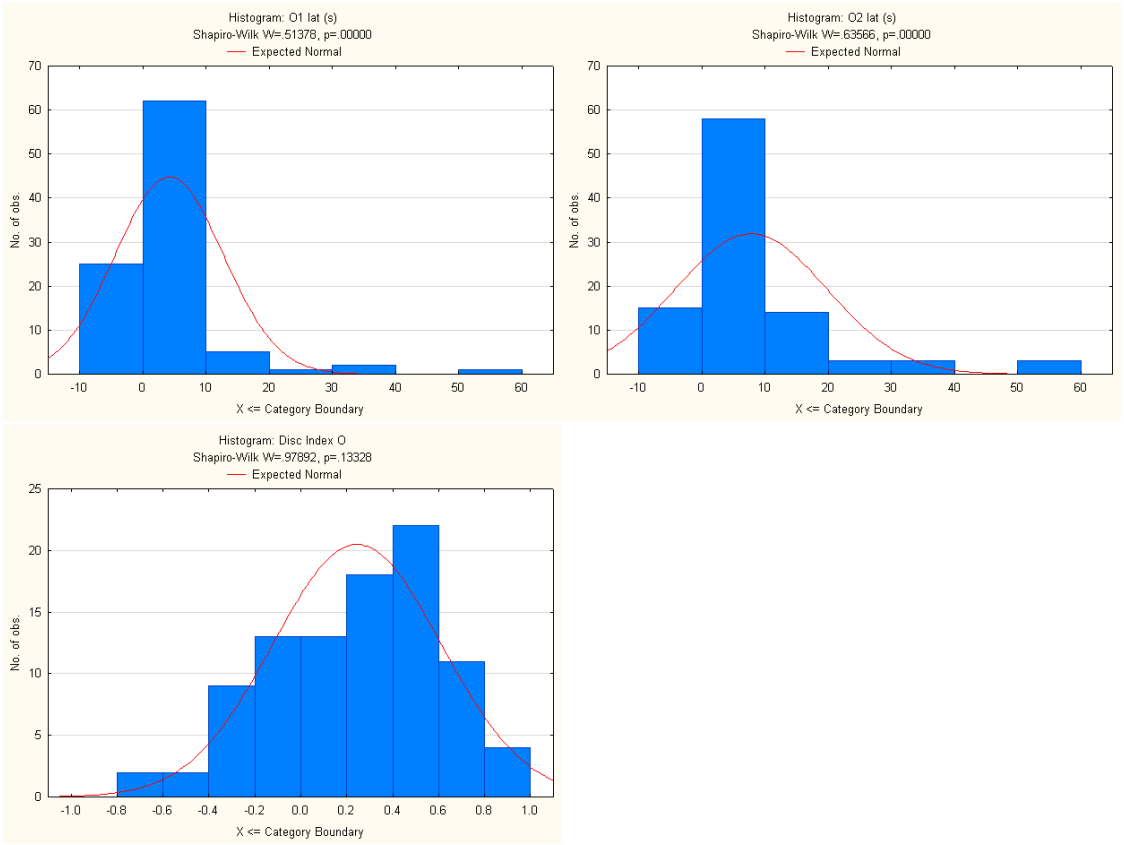


Figure 83- NOR P.3 1 MIN, Histograms 3/3

Table 58- NOR P.3 1 MIN, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
1m Distance (cm)	F	S	24	814.1069	614.2344	967.4491	111.3196
1m Disc Index Q	F	S	24	0.0594	-0.4127	0.6531	0.2569
1m Disc Index O	F	S	24	0.1560	-0.3890	0.7326	0.3070
1m Distance (cm)	F	I	23	744.2565	466.4453	999.7615	150.7033
1m Disc Index Q	F	I	22	0.1731	-0.5287	0.8545	0.3151
1m Disc Index O	F	I	22	0.3375	-0.7315	0.8863	0.3546
1m Distance (cm)	M	S	25	577.3842	65.40353	882.0853	147.6323
1m Disc Index Q	M	S	25	0.1242	-0.40012	0.7565	0.3304
1m Disc Index O	M	S	25	0.2185	-0.64265	0.8745	0.4408
1m Distance (cm)	M	I	24	480.6945	215.2233	750.3322	123.9241
1m Disc Index Q	M	I	23	0.1440	-0.3785	0.7072	0.3221
1m Disc Index O	M	I	23	0.2777	-0.3551	0.9266	0.3416

Variable	Sex_Treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
1m Q1 (s) Raw	FS	24	19.900	10.000	28.600	15.300	22.200
1m Q1 (s) Corrected	FS	24	19.448	9.360	61.941	14.898	25.182
1m Q2 (s)	FS	24	17.600	10.400	33.000	13.400	21.900
1m Q1 freq	FS	24	5.000	3.000	7.000	4.000	5.000
1m Q2 freq	FS	24	5.000	2.000	7.000	4.000	5.000
1m O1 (s) Raw	FS	24	8.400	3.800	16.200	5.900	11.000
1m O1 (s) Corrected	FS	24	9.745	2.727	32.400	7.176	15.284
1m O2 (s)	FS	24	6.600	3.800	15.600	5.200	8.700
1m O1 freq Raw	FS	24	11.000	5.000	26.000	9.500	15.500
1m O1 freq Corrected	FS	24	13.722	4.500	70.000	8.438	20.244
1m O2 freq	FS	24	10.000	4.000	19.000	8.000	13.000
1m O1 lat (s)	FS	24	1.700	0.000	12.400	0.100	3.400
1m O2 lat (s)	FS	24	3.000	0.000	12.200	0.300	7.000
1m Q1 (s) Raw	FI	23	20.400	11.200	28.200	14.200	23.400
1m Q1 (s) Corrected	FI	22	23.490	6.018	163.200	16.577	32.888
1m Q2 (s)	FI	22	16.400	8.800	26.200	11.800	18.800
1m Q1 freq	FI	23	5.000	2.000	7.000	4.000	6.000
1m Q2 freq	FI	23	4.000	0.000	6.000	3.000	5.000
1m O1 (s) Raw	FI	23	8.800	5.800	14.200	7.400	11.800
1m O1 (s) Corrected	FI	22	13.428	1.582	89.600	5.738	23.982
1m O2 (s)	FI	22	5.300	2.600	13.600	4.200	9.200
1m O1 freq Raw	FI	23	10.000	4.000	20.000	9.000	16.000
1m O1 freq Corrected	FI	22	17.261	1.500	48.000	9.333	22.400
1m O2 freq	FI	22	9.500	4.000	22.000	7.000	11.000
1m O1 lat (s)	FI	23	1.600	0.000	12.600	0.200	4.000
1m O2 lat (s)	FI	23	5.400	0.000	60.000	1.200	9.200
1m Q1 (s) Raw	MS	25	20.400	9.4000	40.400	15.800	25.000
1m Q1 (s) Corrected	MS	25	21.242	6.3236	60.652	12.082	27.117
1m Q2 (s)	MS	25	16.400	1.4000	28.200	14.000	20.400
1m Q1 freq	MS	25	4.000	2.0000	7.000	3.000	4.000
1m Q2 freq	MS	25	3.000	1.0000	6.000	3.000	4.000
1m O1 (s) Raw	MS	24	9.200	1.4000	14.800	5.900	11.000
1m O1 (s) Corrected	MS	25	10.400	0.0000	49.636	4.471	22.109
1m O2 (s)	MS	25	7.400	0.0000	13.600	4.000	8.600
1m O1 freq Raw	MS	25	12.000	0.0000	24.000	8.000	15.000
1m O1 freq Corrected	MS	25	14.300	0.0000	54.000	7.500	22.500
1m O2 freq	MS	25	8.000	0.0000	18.000	6.000	14.000
1m O1 lat (s)	MS	25	1.000	0.0000	60.000	0.400	4.800
1m O2 lat (s)	MS	25	6.400	0.0000	60.000	0.600	9.000
1m Q1 (s) Raw	MI	24	19.600	8.200	30.200	13.600	23.500
1m Q1 (s) Corrected	MI	23	26.147	3.156	62.578	17.927	34.987
1m Q2 (s)	MI	23	17.400	6.000	33.000	13.200	23.400
1m Q1 freq	MI	24	3.000	1.000	6.000	2.000	4.000
1m Q2 freq	MI	24	3.000	1.000	5.000	2.000	4.000
1m O1 (s) Raw	MI	24	7.400	3.200	27.000	5.400	10.600
1m O1 (s) Corrected	MI	23	13.608	2.760	47.250	6.612	18.200
1m O2 (s)	MI	23	6.200	1.800	11.800	5.200	7.400
1m O1 freq Raw	MI	24	10.000	5.000	20.000	8.000	13.000
1m O1 freq Corrected	MI	23	13.444	2.188	96.000	6.857	18.667
1m O2 freq	MI	23	8.000	4.000	20.000	6.000	12.000
1m O1 lat (s)	MI	24	0.600	0.000	37.400	0.000	5.700
1m O2 lat (s)	MI	24	3.300	0.000	33.600	0.400	18.900

Table 59- NOR P.3 1 MIN, Distance travelled (cm) statistics

Parametric factorial ANOVA for all sex and housing groups. Followed by Bonferroni post hoc.

Univariate Tests of Significance for Distance (cm) (2.3 P3 NOR Corrected (1 min))
Sigma-restricted parameterization
Effective hypothesis decomposition

Effect	SS	Degr. of Freedom	MS	F	p
Intercept	41038931	1	41038931	2273.116	0.000000
Sex	1500404	1	1500404	83.106	0.000000
Treatment	166269	1	166269	9.210	0.003130
Sex*Treatment	4318	1	4318	0.239	0.625956
Error	1660972	92	18054		

Bonferroni test; variable Distance (cm) (2.3 P3 NOR Corrected (1 min))
Probabilities for Post Hoc Tests
Error: Between MS = 18054., df = 92.000

Cell No.	Sex	{1} 779.92	{2} 530.03
1	F		0.000000
2	M	0.000000	

Bonferroni test; variable Distance (cm) (2.3 P3 NOR Corrected (1 min))
Probabilities for Post Hoc Tests
Error: Between MS = 18054., df = 92.000

Cell No.	Treatment	{1} 693.33	{2} 609.67
1	S		0.002993
2	I	0.002993	

Table 60- NOR P.3 1 MIN, Quadrant 1 vs. Quadrant 2 (s) statistics, corrected data

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

All Groups
Friedman ANOVA and Kendall Coeff. of Concordance (2.3 P3 NOR Corrected (1 min))
ANOVA Chi Sqr. (N = 94, df = 1) = 6.127660 p = .01331
Coeff. of Concordance = .06519 Aver. rank r = .05514

Variable	Average Rank	Sum of Ranks	Mean	Std.Dev.
New Q1 (s)	1.627660	153.0000	26.02307	19.43646
Q2 (s)	1.372340	129.0000	17.54255	6.01601

Aggregate Results
Wilcoxon Matched Pairs Test (2.3 P3 NOR Corrected (1 min))
Marked tests are significant at p <.05000

Pair of Variables	Sex_Treatment	Valid N	T	Z	p-value
New Q1 (s) & Q2 (s)	FS	24	105.0000	1.285714	0.198544
New Q1 (s) & Q2 (s)	FI	22	45.00000	2.645950	0.008147
New Q1 (s) & Q2 (s)	MS	25	97.00000	1.762403	0.078002
New Q1 (s) & Q2 (s)	MI	23	70.00000	2.068217	0.038620

Table 61- NOR P.3 1 MIN, Quadrant 1 vs. Quadrant 2 (number of entries) statistics

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups.

Variable	All Groups Friedman ANOVA and Kendall Coeff. of Concordance (2.3 P3 NOR Corrected (1 min)) ANOVA Chi Sqr. (N = 96, df = 1) = 3.358209 p = .06687 Coeff. of Concordance = .03498 Aver. rank r = .02482			
	Average Rank	Sum of Ranks	Mean	Std.Dev.
Q1 freq	1.578125	151.5000	4.031250	1.356878
Q2 freq	1.421875	136.5000	3.875000	1.363046

Table 62- NOR P.3 1 MIN, Quadrant discrimination index statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for Disc Index Z (2.3 P3 NOR Corrected (1 min)) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	1.469557	1	1.469557	15.54983	0.000159
Sex	0.007499	1	0.007499	0.07935	0.778829
Treatment	0.104369	1	0.104369	1.10436	0.296126
Sex*Treatment	0.051624	1	0.051624	0.54625	0.461777
Error	8.505569	90	0.094506		

Table 63- NOR P.3 1 MIN, Object 1 vs. Object 2 (s) statistics, corrected data

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

Variable	All Groups Friedman ANOVA and Kendall Coeff. of Concordance (2.3 P3 NOR Corrected (1 min)) ANOVA Chi Sqr. (N = 94, df = 1) = 19.88172 p = .00001 Coeff. of Concordance = .21151 Aver. rank r = .20303			
	Average Rank	Sum of Ranks	Mean	Std.Dev.
New O1(s)	1.728723	162.5000	14.98653	13.00974
O2 np (s)	1.271277	119.5000	6.74043	2.91217

Pair of Variables	Aggregate Results Wilcoxon Matched Pairs Test (2.3 P3 NOR Corrected (1 min)) Marked tests are significant at p <.05000				
	Sex_Treatment	Valid N	T	Z	p-value
New O1(s) & O2 np (s)	FS	24	71.00000	2.257143	0.024000
New O1(s) & O2 np (s)	FI	22	18.00000	3.522523	0.000428
New O1(s) & O2 np (s)	MS	24	54.00000	2.742857	0.006091
New O1(s) & O2 np (s)	MI	23	31.00000	3.254400	0.001136

Table 64- NOR P.3 1 MIN, Object 1 vs. Object 2 (number of approaches) statistics, corrected data

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

Variable	All Groups Friedman ANOVA and Kendall Coeff. of Concordance (2.3 P3 NOR Corrected (1 min)) ANOVA Chi Sqr. (N = 94, df = 1) = 15.69565 p = .00007 Coeff. of Concordance = .16698 Aver. rank r = .15802			
	Average Rank	Sum of Ranks	Mean	Std.Dev.
New O1 freq	1.702128	160.0000	17.52060	14.33625
O2 np freq	1.297872	122.0000	9.74468	4.34020

Pair of Varial	Aggregate Results Wilcoxon Matched Pairs Test (2.3 P3 NOR Corrected (1 min)) Marked tests are significant at p <.05000				
	Sex_Treatment	Valid N	T	Z	p-value
New O1 freq	FS	23	59.00000	2.402781	0.016271
New O1 freq	FI	22	29.00000	3.165401	0.001549
New O1 freq	MS	24	61.00000	2.542857	0.010995
New O1 freq	MI	23	55.00000	2.524441	0.011589

Table 65- NOR P.3 1 MIN, Object 1 vs. Object 2 (latency of approach, s) statistics

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups.

Variable	All Groups Friedman ANOVA and Kendall Coeff. of Concordance (Spreadsheet93) ANOVA Chi Sqr. (N = 96, df = 1) = 1.778947 p = .18228 Coeff. of Concordance = .01853 Aver. rank r = .00820			
	Average Rank	Sum of Ranks	Mean	Std.Dev.
O1 Latency (s)	1.432292	137.5000	4.202083	8.54854
O2 Latency (s)	1.567708	150.5000	7.787500	12.01125

Table 66- NOR P.3 1 MIN, Object discrimination index statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for Disc Index O (2.3 P3 NOR Corrected (1 min)) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	5.74185	1	5.741855	42.92895	0.000000
Sex	0.00005	1	0.000047	0.00035	0.985140
Treatment	0.33974	1	0.339745	2.54010	0.114494
Sex*Treatment	0.08772	1	0.087719	0.65583	0.420172
Error	12.03773	90	0.133753		

A.2.3.2 P.3- FIVE MINUTES

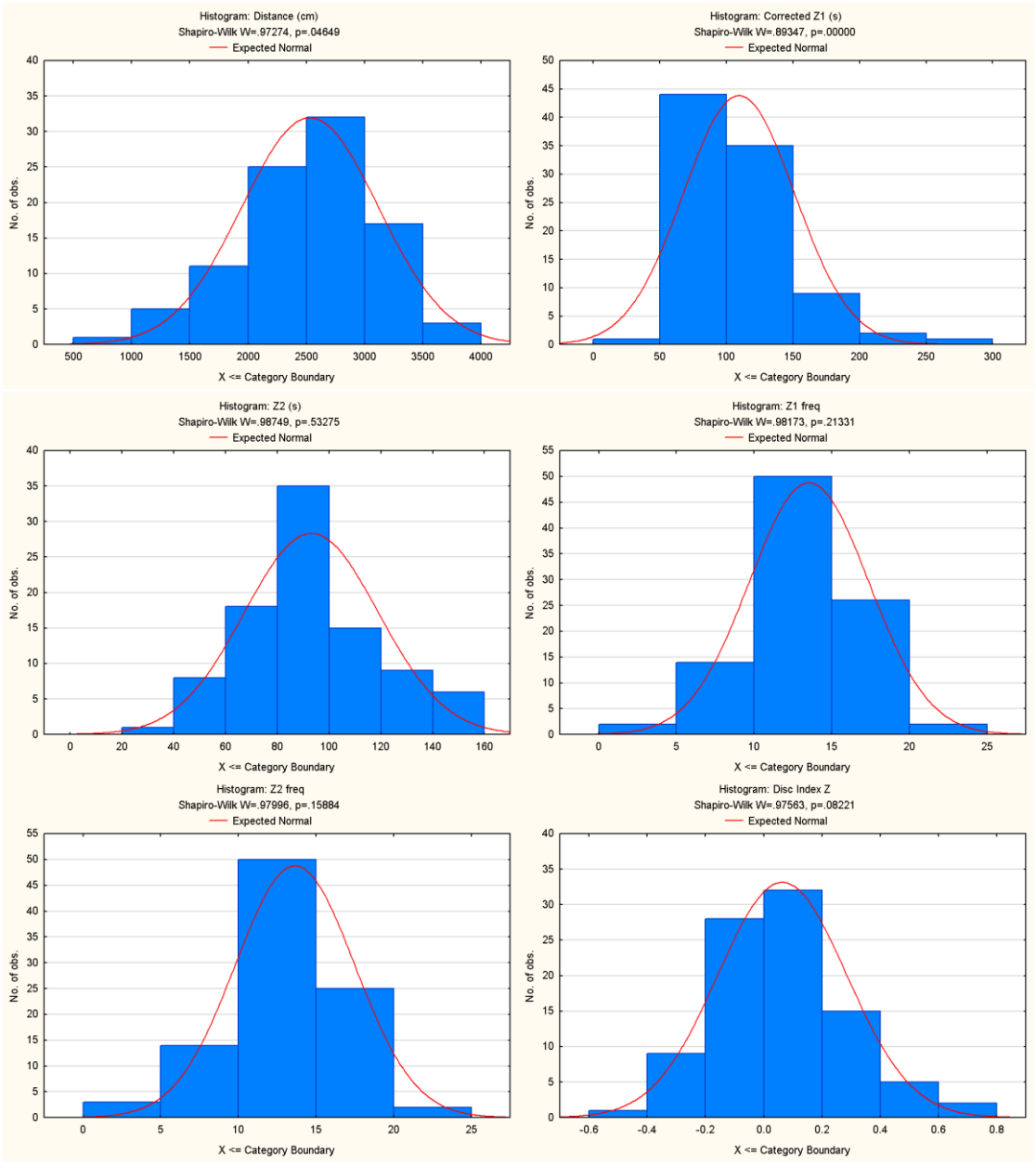


Figure 84- NOR P.3 5 MIN, Histograms 1/2

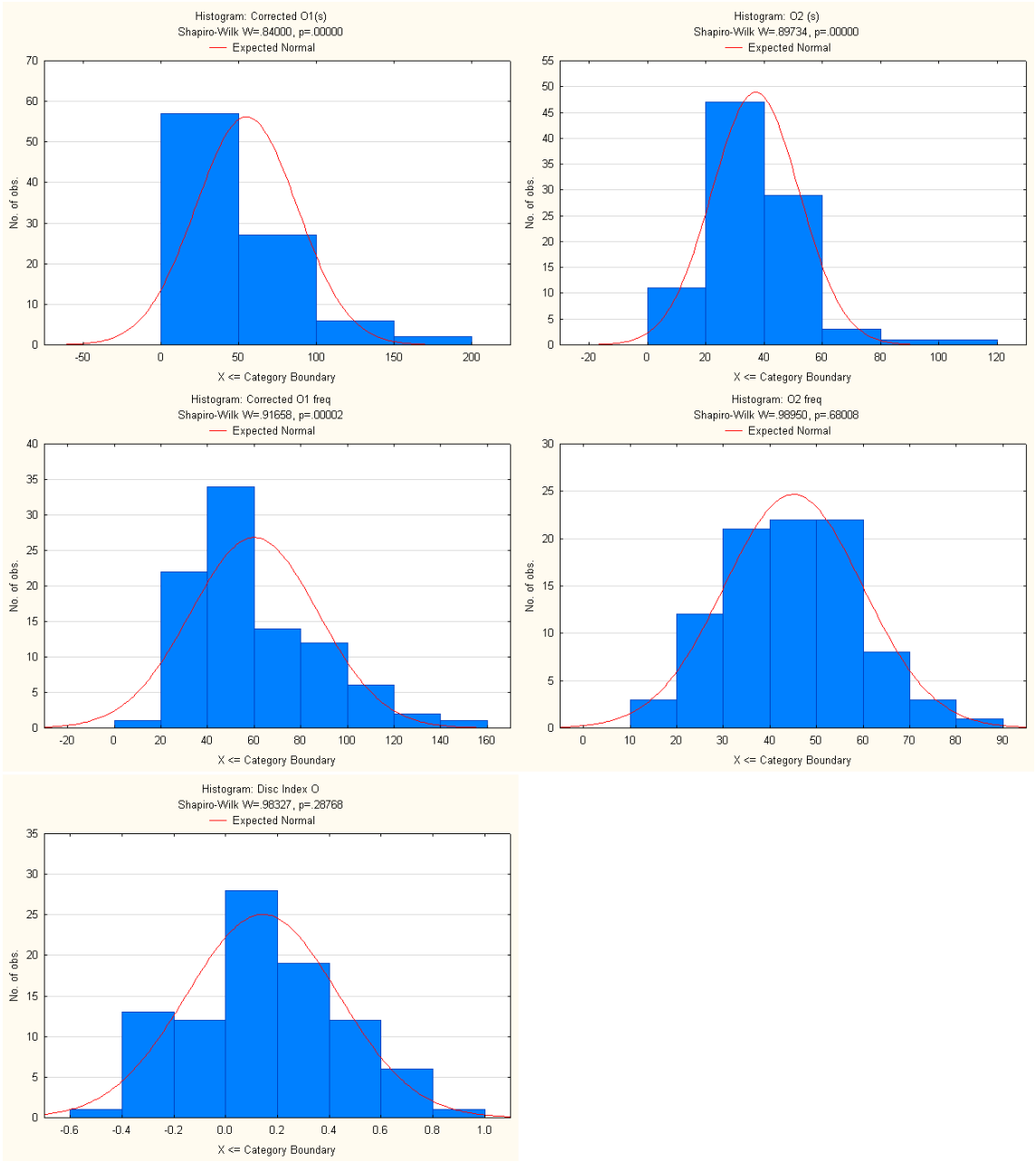


Figure 85- NOR P.3 5 MIN, Histograms 2/2

Table 67- NOR P.3 5 MIN, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
5m Distance (cm)	F	S	24	2883.451	1903.041	3633.545	389.1191
5m Q1 freq	F	S	24	14.792	9.000	20.000	2.7343
5m Q2 freq	F	S	24	15.458	10.000	22.000	3.2834
5m Disc Index Q	F	S	24	0.035	-0.401	0.449	0.2142
5m Disc Index O	F	S	24	0.092	-0.380	0.696	0.3161
5m Distance (cm)	F	I	23	2812.488	1352.637	3555.639	532.9329
5m Q1 freq	F	I	23	15.348	6.000	22.000	3.5369
5m Q2 freq	F	I	23	14.739	5.000	20.000	4.1365
5m Disc Index Q	F	I	22	0.052	-0.148	0.733	0.1941
5m Disc Index O	F	I	22	0.175	-0.291	0.830	0.2572
5m Distance (cm)	M	S	25	2357.388	844.1648	3104.679	486.9378
5m Q1 freq	M	S	25	13.080	6.0000	24.000	3.7072
5m Q2 freq	M	S	25	12.800	8.0000	21.000	2.9861
5m Disc Index Q	M	S	25	0.073	-0.3152	0.523	0.2339
5m Disc Index O	M	S	25	0.126	-0.3651	0.677	0.2990
5m Distance (cm)	M	I	22	2048.886	1133.951	2971.596	530.7705
5m Q1 freq	M	I	22	10.773	4.000	16.000	3.8413
5m Q2 freq	M	I	22	11.455	4.000	18.000	3.8139
5m Disc Index Q	M	I	21	0.095	-0.240	0.732	0.2502
5m Disc Index O	M	I	21	0.187	-0.410	0.765	0.3036

Variable	Sex_Treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
5m Q1 (s) Raw	FS	24	85.0000	57.60000	119.0000	79.00000	103.1000
5m Q1 (s) Corrected	FS	24	101.4618	42.83077	180.8419	77.59769	129.1363
5m Q2 (s)	FS	24	88.2000	53.40000	160.0000	77.90000	117.4000
5m O1 (s) Raw	FS	24	34.8000	22.20000	54.8000	30.30000	43.5000
5m O1 (s) Corrected	FS	24	49.0610	17.24043	187.8024	29.49504	54.7099
5m O2 (s)	FS	24	36.7000	22.80000	110.6000	26.80000	43.8000
5m O1 freq Raw	FS	24	49.5000	25.00000	71.0000	40.00000	58.5000
5m O1 freq Corrected	FS	24	52.3158	27.69231	148.1739	41.90278	91.4023
5m O2 freq	FS	24	48.5000	26.00000	80.0000	37.50000	58.0000
5m Q1 (s) Raw	FI	23	89.60000	61.00000	238.0000	77.00000	103.0000
5m Q1 (s) Corrected	FI	22	94.01653	64.38000	190.7934	74.35323	108.1121
5m Q2 (s)	FI	22	88.80000	29.40000	124.4000	74.80000	99.2000
5m O1 (s) Raw	FI	23	37.40000	25.00000	147.8000	32.20000	45.0000
5m O1 (s) Corrected	FI	22	45.89243	17.35082	167.8633	36.31287	55.9967
5m O2 (s)	FI	22	32.00000	15.20000	70.0000	23.80000	42.2000
5m O1 freq Raw	FI	23	47.00000	25.00000	102.0000	39.00000	59.0000
5m O1 freq Corrected	FI	22	52.46652	30.72917	89.4118	38.47826	68.6538
5m O2 freq	FI	22	43.50000	14.00000	68.0000	35.00000	52.0000
5m Q1 (s) Raw	MS	25	94.00000	60.00000	177.2000	83.60000	106.8000
5m Q1 (s) Corrected	MS	25	97.50268	54.33276	247.3948	77.65512	142.1606
5m Q2 (s)	MS	25	96.80000	43.40000	145.4000	77.80000	108.4000
5m O1 (s) Raw	MS	25	35.00000	10.00000	63.8000	30.80000	42.2000
5m O1 (s) Corrected	MS	25	41.67692	11.95652	134.4149	35.20000	58.4458
5m O2 (s)	MS	25	34.80000	9.80000	65.2000	28.60000	44.8000
5m O1 freq Raw	MS	25	44.00000	7.00000	88.0000	36.00000	54.0000
5m O1 freq Corrected	MS	25	48.78049	6.78462	121.8462	42.48276	71.7391
5m O2 freq	MS	25	47.00000	21.00000	72.0000	33.00000	51.0000
5m Q1 (s) Raw	MI	22	91.6000	38.80000	210.2000	81.00000	105.0000
5m Q1 (s) Corrected	MI	21	121.0032	60.17598	276.9762	90.37170	136.3315
5m Q2 (s)	MI	21	98.2000	42.80000	153.2000	83.00000	109.8000
5m O1 (s) Raw	MI	22	37.2000	16.00000	121.8000	30.20000	48.8000
5m O1 (s) Corrected	MI	21	49.9571	23.16055	147.4993	37.89908	83.4639
5m O2 (s)	MI	21	38.8000	15.60000	65.4000	27.00000	45.4000
5m O1 freq Raw	MI	22	44.5000	18.00000	82.0000	35.00000	50.0000
5m O1 freq Corrected	MI	21	48.3750	21.78947	114.9362	37.56818	72.8000
5m O2 freq	MI	21	42.0000	12.00000	85.0000	38.00000	55.0000

Table 68- NOR P.3 5 MIN, Distance travelled (cm) statistics

Parametric factorial ANOVA for all sex and housing groups, followed by Bonferroni post hoc.

(This variable was non-parametric (p=0.04649) but parametric analyses were used as distance travelled was parametric in all other stages of testing).

Univariate Tests of Significance for Distance (cm) (2 NOR 5Ms P3) Sigma-restricted parameterization Effective hypothesis decomposition					
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	603099442	1	603099442	2555.818	0.000000
Sex	9825751	1	9825751	41.640	0.000000
Treatment	864646	1	864646	3.664	0.058770
Sex*Treatment	237646	1	237646	1.007	0.318290
Error	21237405	90	235971		

Bonferroni test; variable Distance (cm) (2 NOR 5Ms P3) Probabilities for Post Hoc Tests Error: Between MS = 2360E2, df = 90.000			
Cell No.	Sex	{1} 2858.1	{2} 2217.2
1	F		0.000000
2	M	0.000000	

Table 69- NOR P.3 5 MIN, Quadrant 1 vs. Quadrant 2 (s) statistics, corrected data

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups.

Friedman ANOVA and Kendall Coeff. of Concordance (Spreadsheet3) ANOVA Chi Sqr. (N = 92, df = 1) = 2.782609 p = .09529 Coeff. of Concordance = .03025 Aver. rank r = .01959				
Variable	Average Rank	Sum of Ranks	Mean	Std.Dev.
New Q1 (s)	1.586957	146.0000	109.4369	41.85000
Q2(s)	1.413043	130.0000	93.1261	25.88609

Table 70- NOR P.3 5 MIN, Quadrant 1 vs. Quadrant 2 (number of entries) statistics

Parametric repeated measures ANOVA (quadrants) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (Spreadsheet7) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	34722.74	1	34722.74	1585.746	0.000000
Sex	446.54	1	446.54	20.393	0.000019
Treatment	45.16	1	45.16	2.062	0.154439
Sex*Treatment	28.94	1	28.94	1.322	0.253334
Error	1970.71	90	21.90		
ZONE	0.62	1	0.62	0.224	0.637363
ZONE*Sex	0.00	1	0.00	0.001	0.980044
ZONE*Treatment	1.18	1	1.18	0.425	0.515962
ZONE*Sex*Treatment	14.67	1	14.67	5.309	0.023522
Error	248.78	90	2.76		

Bonferroni test; variable DV_1 (Spreadsheet7)
Probabilities for Post Hoc Tests
Error: Between MS = 21.897, df = 90.000

Cell	Sex	{1} 15.141	{2} 12.094
1	F		0.000023
2	M	0.000023	

Bonferroni test; variable DV_1 (Spreadsheet7)
Probabilities for Post Hoc Tests
Error: Between; Within; Pooled MS = 12.331, df = 112.37

Cell	Sex	Treatment	ZONE	{1} 14.826	{2} 15.652	{3} 15.348	{4} 14.739	{5} 13.080	{6} 12.800	{7} 10.913	{8} 11.435
1	F	S	Z1 cp freq		1.000000	1.000000	1.000000	1.000000	1.000000	0.007105	0.039331
2	F	S	Z2 cp freq	1.000000		1.000000	1.000000	0.353118	0.163084	0.000343	0.002430
3	F	I	Z1 cp freq	1.000000	1.000000		1.000000	0.766517	0.376647	0.001094	0.007105
4	F	I	Z2 cp freq	1.000000	1.000000	1.000000		1.000000	1.000000	0.009559	0.051455
5	M	S	Z1 cp freq	1.000000	0.353118	0.766517	1.000000		1.000000	0.976123	1.000000
6	M	S	Z2 cp freq	1.000000	0.163084	0.376647	1.000000	1.000000		1.000000	1.000000
7	M	I	Z1 cp freq	0.007105	0.000343	0.001094	0.009559	0.976123	1.000000		1.000000
8	M	I	Z2 cp freq	0.039331	0.002430	0.007105	0.051455	1.000000	1.000000	1.000000	

Table 71- NOR P.3 5 MIN, Quadrant discrimination index statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for Disc Index Q (Spreadsheet3) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0.366990	1	0.366990	7.370833	0.007977
Sex	0.058588	1	0.058588	1.176706	0.280990
Treatment	0.019206	1	0.019206	0.385751	0.536147
Sex*Treatment	0.000055	1	0.000055	0.001100	0.973615
Error	4.381474	88	0.049789		

Table 72- NOR P.3 5 MIN, Object 1 vs. Object 2 (s) statistics, corrected data

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

Variable	Friedman ANOVA and Kendall Coeff. of Concordance (Spreadsheet3) ANOVA Chi Sqr. (N = 92, df = 1) = 17.39130 p = .00003 Coeff. of Concordance = .18904 Aver. rank r = .18012			
	Average Rank	Sum of Ranks	Mean	Std.Dev.
New O1(s)	1.717391	158.0000	55.05962	32.63320
O2 np (s)	1.282609	118.0000	37.08696	15.01104

Pair of Variables	Aggregate Results Wilcoxon Matched Pairs Test (Spreadsheet3) Marked tests are significant at p <.05000				
	Sex_Treatment	Valid N	T	Z	p-value
New O1(s) & O2 np (s)	FS	23	103.0000	1.064523	0.287092
New O1(s) & O2 np (s)	FI	22	39.00000	2.840744	0.004501
New O1(s) & O2 np (s)	MS	25	99.00000	1.708589	0.087528
New O1(s) & O2 np (s)	MI	22	41.00000	2.775813	0.005507

Table 73- NOR P.3 5 MIN, Object 1 vs. Object 2 (number of approaches) statistics, corrected data

Nonparametric comparison of multiple dependent sample groups, Friedman test for all sex_housing groups. Followed by comparison of two dependant sample groups, Wilcoxon matched pairs test.

Variable	All Groups Friedman ANOVA and Kendall Coeff. of Concordance (Spreadsheet3) ANOVA Chi Sqr. (N = 92, df = 1) = 9.782609 p = .00176 Coeff. of Concordance = .10633 Aver. rank r = .09651			
	Average Rank	Sum of Ranks	Mean	Std.Dev.
New O1 freq	1.663043	153.0000	60.08616	27.36199
O2 np freq	1.336957	123.0000	45.07609	14.87917

Pair of Variables	Aggregate Results Wilcoxon Matched Pairs Test (Spreadsheet3) Marked tests are significant at p <.05000				
	Sex_Treatment	Valid N	T	Z	p-value
New O1 freq & O2 np freq	FS	23	65.00000	2.220292	0.026400
New O1 freq & O2 np freq	FI	22	52.00000	2.418691	0.015577
New O1 freq & O2 np freq	MS	25	88.00000	2.004565	0.045010
New O1 freq & O2 np freq	MI	22	70.00000	1.834309	0.066609

Table 74- NOR P.3 5 MIN, Object discrimination index statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for Disc Index O (Spreadsheet3) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	1.908176	1	1.908176	22.03819	0.000010
Sex	0.027674	1	0.027674	0.31961	0.573279
Treatment	0.161297	1	0.161297	1.86288	0.175773
Sex*Treatment	0.003910	1	0.003910	0.04515	0.832214
Error	7.619479	88	0.086585		

A.2.4 ALL PHASES- DISTANCE TRAVELLED

A.2.4.1 P.1, P.2, P.3- FIRST MINUTE

Table 75- NOR All phases 1 MIN, Distance travelled (cm) statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (Spreadsheet18) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	102838810	1	102838810	4285.042	0.000000
Sex	1769785	1	1769785	73.743	0.000000
Treatment	227486	1	227486	9.479	0.002756
Sex*Treatment	1021	1	1021	0.043	0.837082
Error	2159954	90	23999		
PHASE	2156888	2	1078444	110.376	0.000000
PHASE*Sex	385780	2	192890	19.742	0.000000
PHASE*Treatment	15317	2	7659	0.784	0.458204
PHASE*Sex*Treatment	31855	2	15928	1.630	0.198778
Error	1758718	180	9771		

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet18) Probabilities for Post Hoc Tests Error: Between MS = 23999., df = 90.000		
	Sex	{1} 685.03	{2} 526.52
1	F		0.000000
2	M	0.000000	

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet18) Probabilities for Post Hoc Tests Error: Between MS = 23999., df = 90.000		
	Treatment	{1} 631.43	{2} 574.33
1	S		0.002650
2	I	0.002650	

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet18) Probabilities for Post Hoc Tests Error: Within MS = 9770.7, df = 180.00			
	PHASE	{1} 481.52	{2} 674.00	{3} 656.76
1	1 MIN P1 Distance (cm)		0.000000	0.000000
2	1 MIN P2 Distance (cm)	0.00		0.700254
3	1 MIN P3 Distance (cm)	0.00	0.700254	

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet18) Probabilities for Post Hoc Tests Error: Between; Within; Pooled MS = 14514., df = 222.48							
	Sex	PHASE	{1} 519.54	{2} 749.50	{3} 786.06	{4} 445.09	{5} 601.64	{6} 532.84
1	F	1 MIN P1 Distance (cm)		0.000000	0.000000	**0.045764**	0.016731	1.000000
2	F	1 MIN P2 Distance (cm)	0.000000		1.000000	0.000000	**0.000000**	0.000000
3	F	1 MIN P3 Distance (cm)	0.000000	1.000000		0.000000	0.000000	**0.000000**
4	M	1 MIN P1 Distance (cm)	0.045764	0.000000	0.000000		0.000000	0.000342
5	M	1 MIN P2 Distance (cm)	0.016731	0.000000	0.000000	0.000000		0.012039
6	M	1 MIN P3 Distance (cm)	1.000000	0.000000	0.000000	0.000342	0.012039	

A.2.4.2 P.1, P.2, P.3- FIVE MINUTES

Table 76- NOR All phases 5 MIN, Distance travelled (cm) statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (Spreadsheet18) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	2.163133E+09	1	2.163133E+09	5924.948	0.000000
Sex	1.416403E+07	1	1.416403E+07	38.796	0.000000
Treatment	2.022288E+05	1	2.022288E+05	0.554	0.458705
Sex*Treatment	8.954924E+04	1	8.954924E+04	0.245	0.621652
Error	3.212782E+07	88	3.650889E+05		
PHASE	8.779394E+06	2	4.389697E+06	39.771	0.000000
PHASE*Sex	2.859056E+06	2	1.429528E+06	12.952	0.000006
PHASE*Treatment	3.622758E+05	2	1.811379E+05	1.641	0.196712
PHASE*Sex*Treatment	4.082102E+05	2	2.041051E+05	1.849	0.160405
Error	1.942580E+07	176	1.103739E+05		

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet18) Probabilities for Post Hoc Tests Error: Between MS = 3651E2, df = 88.000 Sex	{1} 3030.4	{2} 2579.4
1	F		0.000000
2	M	0.000000	

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet18) Probabilities for Post Hoc Tests Error: Within MS = 1104E2, df = 176.00 PHASE	{1} 2896.7	{2} 2952.7	{3} 2550.5
1	5 MIN P1 Distance (cm)		0.764148	0.000000
2	5 MIN P2 Distance (cm)	0.764148		0.000000
3	5 MIN P3 Distance (cm)	0.000000	0.000000	

Cell No.	Bonferroni test; variable DV_1 (Spreadsheet18) Probabilities for Post Hoc Tests Error: Between; Within; Pooled MS = 1953E2, df = 191.57 Sex	PHASE	{1} 2986.1	{2} 3227.2	{3} 2877.9	{4} 2811.2	{5} 2690.0	{6} 2237.0
1	F	5 MIN P1 Distance (cm)		0.010842	1.000000	0.887607	0.023090	0.000000
2	F	5 MIN P2 Distance (cm)	0.010842		0.000022	0.000167	**0.000000**	0.000000
3	F	5 MIN P3 Distance (cm)	1.000000	0.000022		1.000000	0.641411	**0.000000**
4	M	5 MIN P1 Distance (cm)	0.887607	0.000167	1.000000		1.000000	0.000000
5	M	5 MIN P2 Distance (cm)	0.023090	0.000000	0.641411	1.000000		0.000000
6	M	5 MIN P3 Distance (cm)	0.000000	0.000000	0.000000	0.000000	0.000000	

A.3 IN-VITRO SUPERFUSION

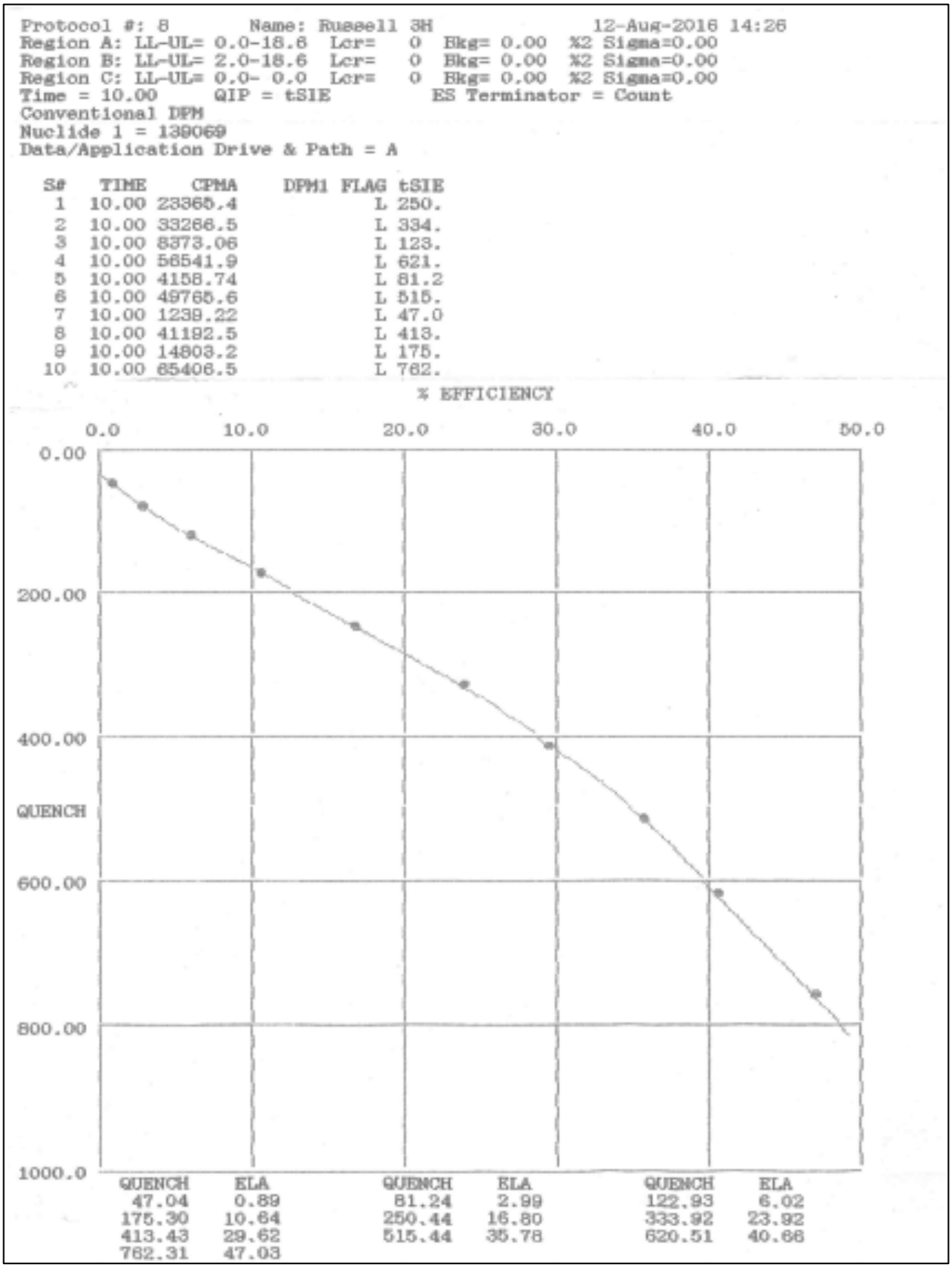


Figure 86- Superfusion standard curve

Standard decay curve generated from quenched tritium samples for superfusion analysis.

A.3.1 SF 1- GLUTAMATE-STIMULATED [^{3}H]NE RELEASE IN HIPPOCAMPUS AND PREFRONTAL CORTEX

Table 77- SF1, Data

Rat #	Sex	Treatment	Sex_Treatment	HC Stim 1	HC Stim 2	HC Stim 3	PFC Stim 1	PFC Stim 2	PFC Stim 3
31	M	I	MI	0.3223	0.1274	-0.0194	0.2618	0.4037	0.1424
27	M	S	MS	0.4467	0.2383	0.1491	0.4542	0.4055	0.3158
33	F	S	FS	0.4650	0.1532	0.2683	0.4239	0.3722	0.4629
37	F	I	FI	0.8354	0.3502	0.7505	0.8286	0.3335	0.1602
38	F	I	FI	0.2837	0.2563	0.1554	0.6506	0.2593	0.1835
34	F	S	FS	0.3101	0.1874	0.0577	0.7017	0.0902	0.0528
41	M	S	MS	0.2483	0.0587	0.2673	0.2067	-0.1171	0.0963
45	M	I	MI	0.1437	0.0689	0.0718	0.2541	0.0205	-0.0144
46	M	I	MI	0.3770	0.1307	0.0444	0.3898	0.1195	0.0023
42	M	S	MS	0.4555	0.2140	0.0243	0.2890	0.1988	0.0797
49	F	S	FS	0.1038	0.1812	0.1303	0.1991	0.1912	0.0645
53	F	I	FI	0.2440	0.1517	0.2577	0.1708	0.3655	0.1851
61	M	I	MI	0.5785	0.0346	-0.0301	0.3402	0.1004	-0.0133
57	M	S	MS	0.6683	0.0838	0.0654	0.3678	0.0291	0.0307
58	M	S	MS	0.6143	0.1211	0.0539	0.5150	0.0807	0.0490
62	M	I	MI	0.6232	0.0317	0.1039	0.5275	0.1353	0.0818
73	M	S	MS	0.4074	0.0477	0.0433	0.5228	0.1971	0.0367
77	M	I	MI	0.6362	0.0939	0.0397	0.3438	0.0623	-0.0012
78	M	I	MI	0.5642	0.0916	0.0780	0.2516	0.0512	-0.0254
74	M	S	MS	0.5853	0.1179	0.0674	0.2884	0.0214	0.0427
75	M	S	MS	0.5688	0.1364	0.0553	0.4441	0.0767	0.1133
79	M	I	MI	0.5849	0.1307	0.0970	0.4805	0.0605	0.0571
77.1	F	I	FI	0.5643	0.1875	0.1512	0.4077	0.0937	0.0788
73.1	F	S	FS	0.7034	0.1642	0.1374	0.5820	0.1588	0.0841
81	F	S	FS	0.8135	0.0898	0.0496	0.4547	0.1592	0.0196
85	F	I	FI	0.5211	0.1245	0.0468	0.3031	0.0846	0.0331
86	F	I	FI	0.4627	-0.0274	0.0974	0.4958	0.1163	0.0798
82	F	S	FS	0.6285	0.1513	0.1371	0.4155	0.1293	0.0008
83	F	S	FS	0.3882	0.0673	0.0502	0.3600	0.0914	0.0930
88	F	I	FI	0.5699	0.1828	0.1029	0.2738	-0.0441	-0.0030

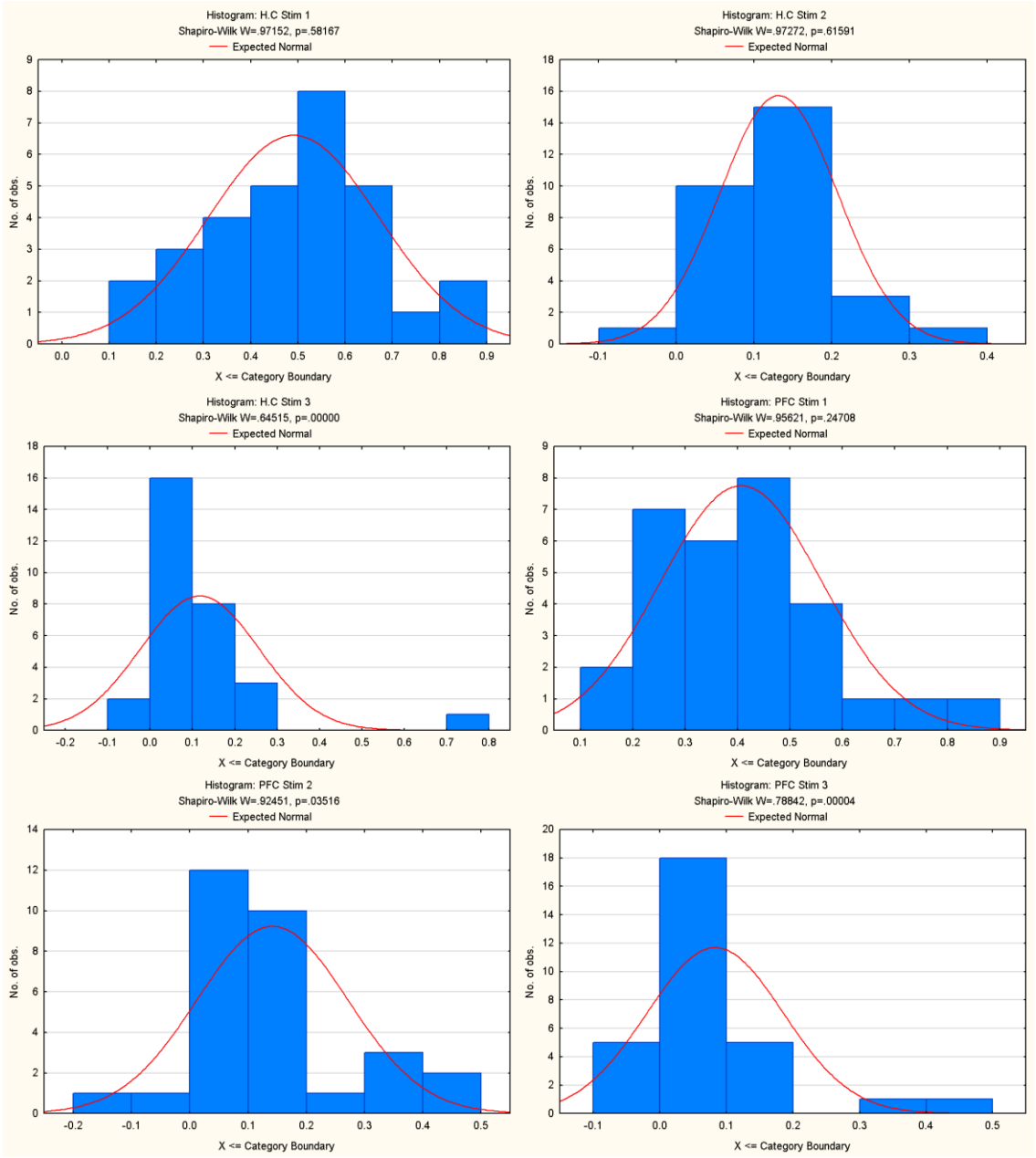


Figure 87- SF1, Histograms

Table 78- SF1, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
H.C Stim 1	M	I	8	0.478747	0.143673	0.636191	0.177793
H.C Stim 2	M	I	8	0.088694	0.031715	0.130716	0.040759
H.C Stim 3	M	I	8	0.048171	-0.030096	0.103875	0.050308
PFC Stim 1	M	I	8	0.356171	0.251630	0.527501	0.104611
PFC Stim 2	M	I	8	0.119196	0.020491	0.403737	0.121075
PFC Stim 3	M	I	8	0.028669	-0.025376	0.142369	0.059387
H.C Stim 1	M	S	8	0.499333	0.248328	0.668334	0.136352
H.C Stim 2	M	S	8	0.127227	0.047652	0.238303	0.068642
H.C Stim 3	M	S	8	0.090761	0.024323	0.267320	0.080224
PFC Stim 1	M	S	8	0.385994	0.206730	0.522838	0.116365
PFC Stim 2	M	S	8	0.111516	-0.117072	0.405508	0.156159
PFC Stim 3	M	S	8	0.095528	0.030699	0.315759	0.093870
H.C Stim 1	F	S	7	0.487516	0.103816	0.813485	0.245557
H.C Stim 2	F	S	7	0.142057	0.067336	0.187360	0.045838
H.C Stim 3	F	S	7	0.118646	0.049624	0.268256	0.077863
PFC Stim 1	F	S	7	0.448128	0.199141	0.701676	0.160035
PFC Stim 2	F	S	7	0.170343	0.090208	0.372227	0.096426
PFC Stim 3	F	S	7	0.111111	0.000826	0.462903	0.158598
H.C Stim 1	F	I	7	0.497291	0.243974	0.835372	0.198152
H.C Stim 2	F	I	7	0.175098	-0.027423	0.350221	0.116619
H.C Stim 3	F	I	7	0.223119	0.046801	0.750473	0.241669
PFC Stim 1	F	I	7	0.447218	0.170807	0.828635	0.230225
PFC Stim 2	F	I	7	0.172698	-0.044059	0.365464	0.149834
PFC Stim 3	F	I	7	0.102498	-0.003000	0.185059	0.074969

Table 79- SF1, HC Stim 1 vs. 2 vs. 3 statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (4.1 SF 1) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	5.513201	1	5.513201	208.0483	0.000000
Sex	0.060103	1	0.060103	2.2681	0.144119
Treatment	0.001293	1	0.001293	0.0488	0.826925
Sex*Treatment	0.038578	1	0.038578	1.4558	0.238465
Error	0.688990	26	0.026500		
STIM	2.640702	2	1.320351	80.9472	0.000000
STIM*Sex	0.035910	2	0.017955	1.1008	0.340240
STIM*Treatment	0.006130	2	0.003065	0.1879	0.829247
STIM*Sex*Treatment	0.013077	2	0.006538	0.4009	0.671802
Error	0.848185	52	0.016311		

Cell No.	Bonferroni test; variable DV_1 (4.1 SF 1) Probabilities for Post Hoc Tests Error: Within MS = .01631, df = 52.000 STIM	{1} .49061	{2} .13158	{3} .11679
1	H.C Stim 1		0.000000	0.000000
2	H.C Stim 2	0.000000		1.000000
3	H.C Stim 3	0.000000	1.000000	

Table 80- SF1, PFC Stim 1 vs. 2 vs. 3 statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (4.1 SF 1) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	4.043050	1	4.043050	142.1483	0.000000
Sex	0.078381	1	0.078381	2.7558	0.108918
Treatment	0.005755	1	0.005755	0.2023	0.656575
Sex*Treatment	0.004167	1	0.004167	0.1465	0.705008
Error	0.739504	26	0.028442		
STIM	1.789811	2	0.894905	72.8942	0.000000
STIM*Sex	0.003896	2	0.001948	0.1587	0.853702
STIM*Treatment	0.006829	2	0.003415	0.2781	0.758316
STIM*Sex*Treatment	0.003779	2	0.001890	0.1539	0.857728
Error	0.638392	52	0.012277		

Cell No.	Bonferroni test; variable DV_1 (4.1 SF 1) Probabilities for Post Hoc Tests Error: Within MS = .01228, df = 52.000 STIM	{1} .40682	{2} .14157	{3} .08296
1	PFC Stim 1		0.000000	0.000000
2	PFC Stim 2	0.000000		0.136722
3	PFC Stim 3	0.000000	0.136722	

Table 81- SF1, Stim 1 HC vs. PFC statistics

Parametric repeated measures ANOVA (brain areas) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (4.1 SF 1) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	12.09867	1	12.09867	278.6481	0.000000
Sex	0.02387	1	0.02387	0.5497	0.465100
Treatment	0.00161	1	0.00161	0.0371	0.848755
Sex*Treatment	0.00328	1	0.00328	0.0755	0.785630
Error	1.12890	26	0.04342		
AREA	0.09881	1	0.09881	5.5491	0.026311
AREA*Sex	0.02002	1	0.02002	1.1243	0.298756
AREA*Treatment	0.00037	1	0.00037	0.0208	0.886429
AREA*Sex*Treatment	0.00000	1	0.00000	0.0001	0.991706
Error	0.46297	26	0.01781		

Cell No.	Bonferroni test; variable DV_1 (4.1 SF 1) Probabilities for Post Hoc Tests Error: Within MS = .01781, df = 26.000 AREA	{1} .49061	{2} .40682
1	H.C Stim 1		0.022220
2	PFC Stim 1	0.022220	

Table 82- SF1, Stim 2 HC vs. PFC statistics

Parametric repeated measures ANOVA (brain areas) for all sex and housing groups.

Effect	Repeated Measures Analysis of Variance (4.1 SF 1) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	1.143400	1	1.143400	71.80355	0.000000
Sex	0.042569	1	0.042569	2.67325	0.114099
Treatment	0.000019	1	0.000019	0.00121	0.972516
Sex*Treatment	0.004096	1	0.004096	0.25724	0.616295
Error	0.414024	26	0.015924		
AREA	0.001544	1	0.001544	0.21290	0.648341
AREA*Sex	0.000115	1	0.000115	0.01584	0.900822
AREA*Treatment	0.000225	1	0.000225	0.03102	0.861551
AREA*Sex*Treatment	0.005519	1	0.005519	0.76085	0.391047
Error	0.188597	26	0.007254		

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Table 83- SF1, Stim 3 HC vs. PFC statistics

Parametric repeated measures ANOVA (brain areas) for all sex and housing groups.

Effect	Repeated Measures Analysis of Variance (4.1 SF 1) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0.625285	1	0.625285	32.73226	0.000005
Sex	0.079714	1	0.079714	4.17283	0.051338
Treatment	0.000172	1	0.000172	0.00902	0.925052
Sex*Treatment	0.039342	1	0.039342	2.05947	0.163184
Error	0.496678	26	0.019103		
AREA	0.019057	1	0.019057	2.21291	0.148889
AREA*Sex	0.012007	1	0.012007	1.39428	0.248373
AREA*Treatment	0.017609	1	0.017609	2.04479	0.164636
AREA*Sex*Treatment	0.007362	1	0.007362	0.85495	0.363659
Error	0.223900	26	0.008612		

A.3.2 SF 2- GLUTAMATE-STIMULATED [^{3}H]NE RELEASE IN HIPPOCAMPUS IN PRESENCE OF MK-801 AND/OR CNQX

Table 84- SF2, Data

Rat #	Sex	Treatment	Sex_Treatment	CONT Stim 1	CONT Stim 2	CONT Stim 3	CNQX Stim 1	CNQX Stim 2	CNQX Stim 3	MK-801 Stim 1	MK-801 Stim 2	MK-801 Stim 3	C+M Stim 1	C+M Stim 2	C+M Stim 3
65	F	S	FS	0.3356	0.0669	0.0519	0.2477	0.0728	-0.1129	0.3734	-0.0448	0.0624	0.1980	-0.0104	0.0171
69	F	I	FI	0.4736	0.0948	0.0889	0.1229	-0.0127	0.0539	0.5752	0.1269	0.0653	0.3443	0.0916	0.0303
70	F	I	FI	0.2890	0.1051	0.0125	0.1755	0.0532	0.0348	0.2296	0.1178	0.0732	0.3027	0.0734	-0.0190
66	F	S	FS	0.5541	0.1235	0.0522	0.3942	0.0735	0.0469	0.1881	0.1385	0.0310	0.2968	0.1310	0.1099
85.1	M	I	MI	0.3324	0.1116	0.0364	0.1522	-0.0071	-0.0013	0.3576	0.1334	0.0438	0.3108	0.1440	-0.0462
81.1	M	S	MS	0.5968	0.2882	0.0901	0.2408	-0.0272	0.0275	0.1163	-0.0399	0.4778	0.4495	0.0427	0.0254
89	M	S	MS	0.3578	0.1148	-0.0980	0.3996	0.0850	-0.3204	0.2056	0.0961	0.0422	0.2798	0.2548	-0.1193
92	M	I	MI	0.2310	-0.0801	-0.0133	0.3005	0.1143	0.0063	0.1140	-0.0054	0.1448	0.1793	-0.0840	0.0032
93	M	I	MI	0.2404	-0.0760	0.0142	0.2220	0.0477	0.1147	0.1085	0.0896	-0.0838	0.0747	-0.1871	0.0643
90	M	S	MS	0.2348	0.1051	0.0522				0.4863	0.1046	0.0916	0.2092	0.0795	-0.1481
91	M	S	MS	0.2798	0.1289	0.0017	0.2687	0.0719	0.0731	0.0164	0.1001	0.0817	0.1722	0.0738	-0.0427
94	M	I	MI	0.0807	0.1688	-0.0776	0.1297	0.0887	0.0037	0.2536	0.4893	0.0680	0.2262	0.0360	0.0221
92.1	F	I	FI	0.4484	0.1036	-0.1108	0.3659	0.0639	0.0475	0.1714	0.1779	0.1405	0.1749	0.0736	-0.0027
89.1	F	S	FS	0.5840	0.0862	0.0746	0.5041	0.2217	0.0645	0.4932	0.2482	0.0661	0.3665	0.1110	-0.0035
90.1	F	S	FS	0.2807	0.2115	0.0467	0.3354	0.1097	-0.2109	0.5344	0.1989	0.1210	0.2277	0.0211	0.0587
93.1	F	I	FI	0.2951	0.1391	0.1091	0.7687	0.3666	0.1495	0.2691	0.0922	0.0462	0.2754	0.0111	0.0258
94.1	F	I	FI	0.2549	0.1064	0.0132	0.2901	0.0733	0.1068	0.3819	0.1990	-0.1078	0.0890	0.0968	-0.0505
91.1	F	S	FS	0.2722	0.2286	0.0096	0.3592	0.0425	-0.0214	0.1606	-0.0459	0.0698	0.3259	0.0970	0.0792
95	M	S	MS	0.1875	-0.0690	0.0308	0.4023	-0.0664	0.0000	0.2694	0.0664	-0.1462	0.2206	0.0051	0.0038
97	M	I	MI	0.2343	0.0695	0.0529	0.1273	-0.0287	-0.0460	0.1610	0.0128	0.0413	0.2587	0.1299	0.0928
96.1	M	I	MI	0.2940	0.0180	0.1189	0.0512	0.0419	-0.0517	0.0357	-0.0082	-0.0187	0.2338	-0.0059	0.0124
95.1	M	S	MS	0.1301	-0.0350	-0.0961	0.0771	0.0656	-0.1776	0.3939	0.1025	-0.1988	0.5778	0.0496	0.0692
100	F	I	FI	0.2959	-0.0124	-0.0038	0.4078	0.0051	-0.0942	0.4723	0.0428	0.0453	0.2023	-0.1839	-0.0780
99	F	S	FS	0.2747	0.0789	0.1338	0.2545	0.0153	0.0049	0.0438	0.0198	-0.0952	0.2999	0.1000	-0.0309

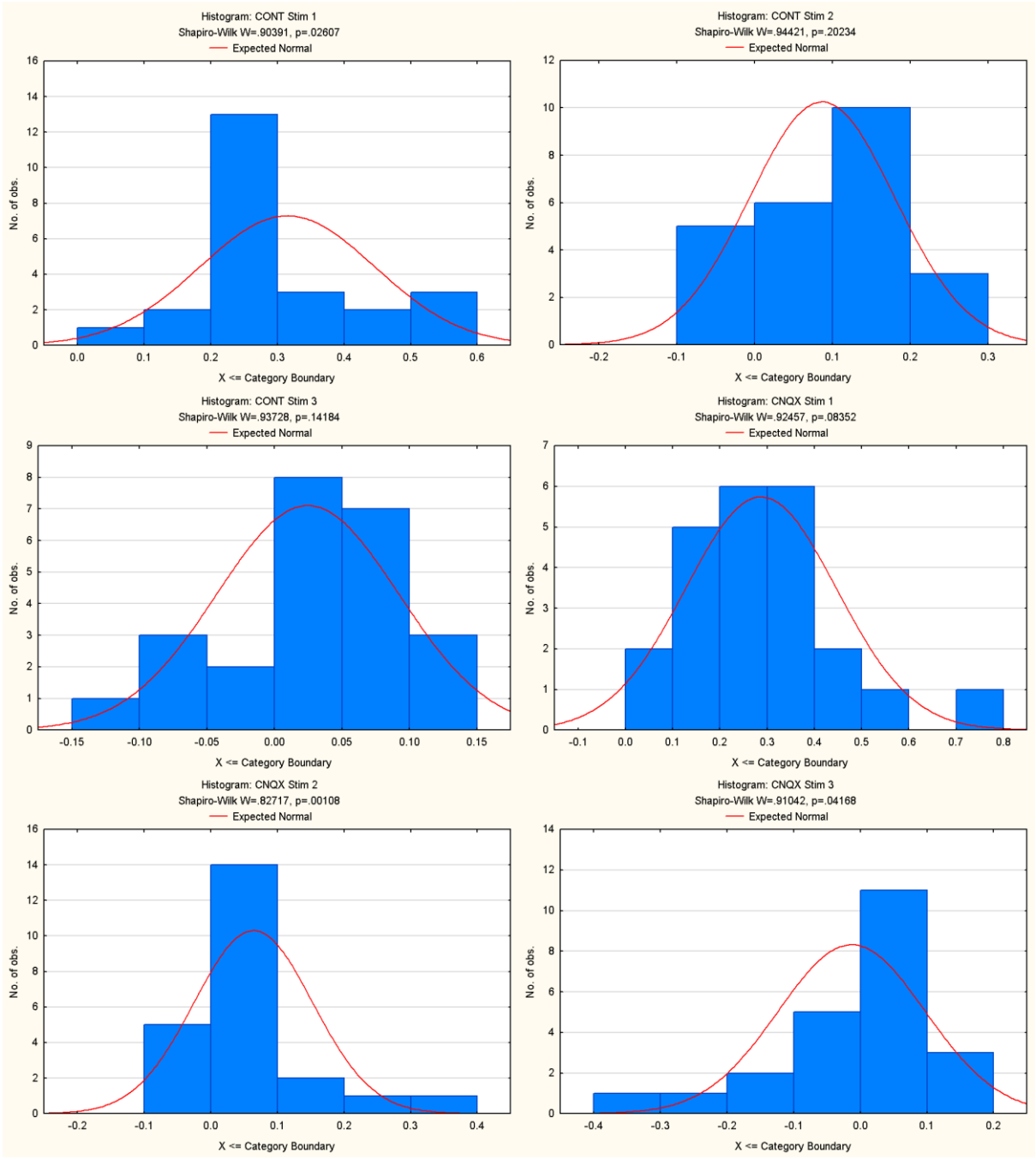


Figure 88- SF2, Histograms 1/2

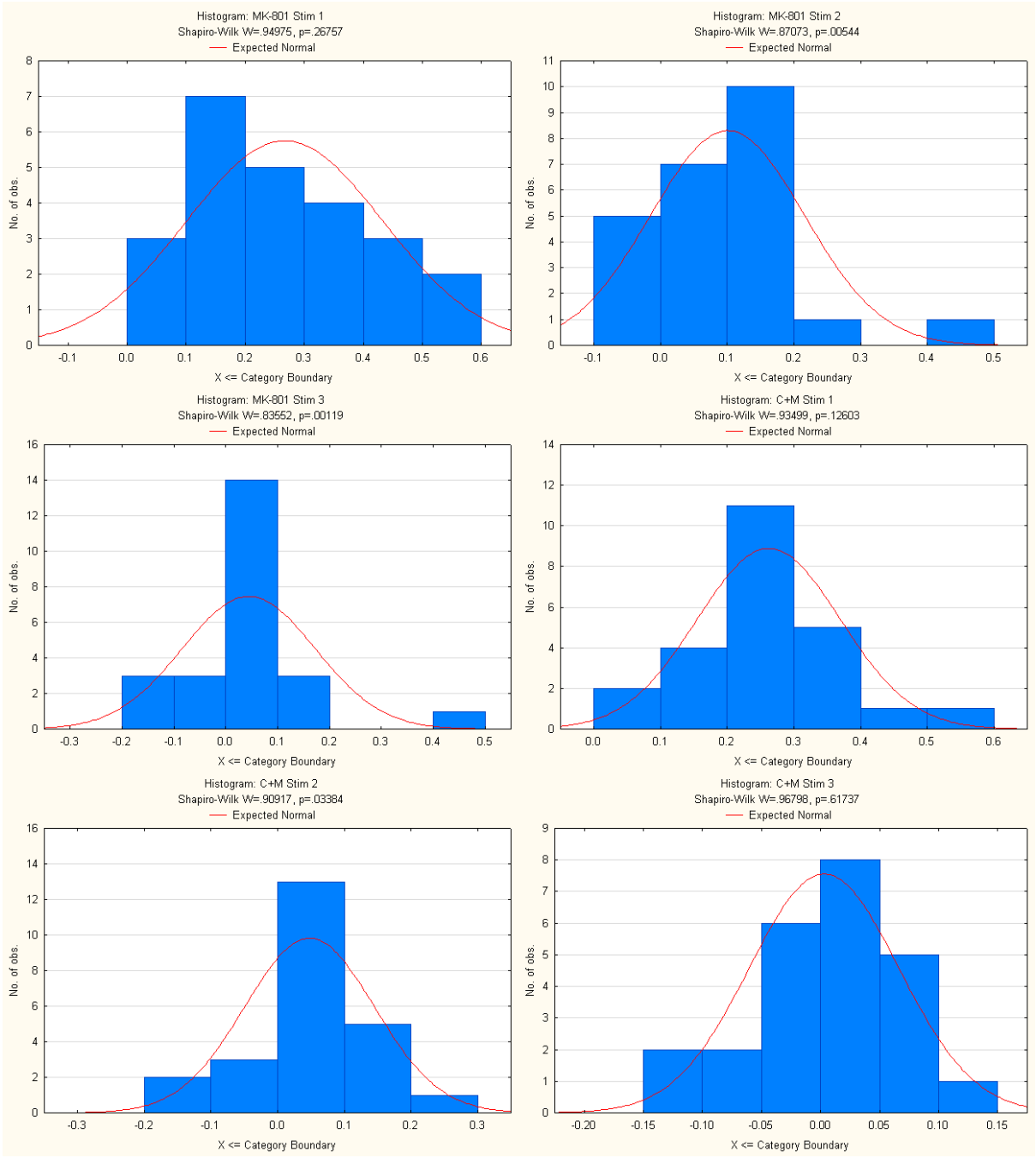


Figure 89- SF2, Histograms 2/2

Table 85- SF2, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
CONT Stim 1	F	S	6	0.384	0.272	0.584	0.146
CONT Stim 2	F	S	6	0.133	0.067	0.229	0.071
CONT Stim 3	F	S	6	0.061	0.010	0.134	0.041
CNQX Stim 1	F	S	6	0.349	0.248	0.504	0.095
CNQX Stim 2	F	S	6	0.089	0.015	0.222	0.072
CNQX Stim 3	F	S	6	-0.038	-0.211	0.064	0.105
MK-801 Stim 1	F	S	6	0.299	0.044	0.534	0.198
MK-801 Stim 2	F	S	6	0.086	-0.046	0.248	0.127
MK-801 Stim 3	F	S	6	0.042	-0.095	0.121	0.073
C+M Stim 1	F	S	6	0.286	0.198	0.366	0.062
C+M Stim 2	F	S	6	0.075	-0.010	0.131	0.056
C+M Stim 3	F	S	6	0.038	-0.031	0.110	0.053
CONT Stim 1	F	I	6	0.343	0.255	0.474	0.093
CONT Stim 2	F	I	6	0.089	-0.012	0.139	0.052
CONT Stim 3	F	I	6	0.018	-0.111	0.109	0.078
CNQX Stim 1	F	I	6	0.355	0.123	0.769	0.230
CNQX Stim 2	F	I	6	0.092	-0.013	0.367	0.139
CNQX Stim 3	F	I	6	0.050	-0.094	0.150	0.083
MK-801 Stim 1	F	I	6	0.350	0.171	0.575	0.155
MK-801 Stim 2	F	I	6	0.126	0.043	0.199	0.057
MK-801 Stim 3	F	I	6	0.044	-0.108	0.141	0.082
C+M Stim 1	F	I	6	0.231	0.089	0.344	0.094
C+M Stim 2	F	I	6	0.027	-0.184	0.097	0.108
C+M Stim 3	F	I	6	-0.016	-0.078	0.030	0.043
CONT Stim 1	M	I	6	0.235	0.081	0.332	0.086
CONT Stim 2	M	I	6	0.035	-0.080	0.169	0.101
CONT Stim 3	M	I	6	0.022	-0.078	0.119	0.066
CNQX Stim 1	M	I	6	0.164	0.051	0.301	0.086
CNQX Stim 2	M	I	6	0.043	-0.029	0.114	0.054
CNQX Stim 3	M	I	6	0.004	-0.052	0.115	0.060
MK-801 Stim 1	M	I	6	0.172	0.036	0.358	0.116
MK-801 Stim 2	M	I	6	0.119	-0.008	0.489	0.190
MK-801 Stim 3	M	I	6	0.033	-0.084	0.145	0.078
C+M Stim 1	M	I	6	0.214	0.075	0.311	0.081
C+M Stim 2	M	I	6	0.005	-0.187	0.144	0.127
C+M Stim 3	M	I	6	0.025	-0.046	0.093	0.049
CONT Stim 1	M	S	6	0.298	0.130	0.597	0.166
CONT Stim 2	M	S	6	0.089	-0.069	0.288	0.128
CONT Stim 3	M	S	6	-0.003	-0.098	0.090	0.078
CNQX Stim 1	M	S	5	0.278	0.077	0.402	0.134
CNQX Stim 2	M	S	5	0.026	-0.066	0.085	0.068
CNQX Stim 3	M	S	5	-0.079	-0.320	0.073	0.165
MK-801 Stim 1	M	S	6	0.248	0.016	0.486	0.174
MK-801 Stim 2	M	S	6	0.072	-0.040	0.105	0.056
MK-801 Stim 3	M	S	6	0.058	-0.199	0.478	0.239
C+M Stim 1	M	S	6	0.318	0.172	0.578	0.161
C+M Stim 2	M	S	6	0.084	0.005	0.255	0.088
C+M Stim 3	M	S	6	-0.035	-0.148	0.069	0.085

Table 86- SF2, Control condition Stim 1 vs. 2 vs. 3 statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (SF 2x) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	1.452112	1	1.452112	104.6700	0.000000
Sex	0.061936	1	0.061936	4.4644	0.047376
Treatment	0.023745	1	0.023745	1.7115	0.205623
Sex*Treatment	0.000664	1	0.000664	0.0478	0.829071
Error	0.277465	20	0.013873		
STIM	1.122229	2	0.561114	72.4584	0.000000
STIM*Sex	0.013940	2	0.006970	0.9001	0.414609
STIM*Treatment	0.006707	2	0.003354	0.4331	0.651519
STIM*Sex*Treatment	0.007211	2	0.003605	0.4656	0.631131
Error	0.309758	40	0.007744		

Cell No.	Bonferroni test; variable DV_1 (SF 2x) Probabilities for Post Hoc Tests Error: Between MS = .01387, df = 20.000 Sex	{1} .17134	{2} .11269
1	F		0.047376
2	M	0.047376	

Cell No.	Bonferroni test; variable DV_1 (SF 2x) Probabilities for Post Hoc Tests Error: Within MS = .00774, df = 40.000 STIM	{1} .31491	{2} .08654	{3} .02459
1	CONT Stim 1		0.000000	0.000000
2	CONT Stim 2	0.000000		0.057834
3	CONT Stim 3	0.000000	0.057834	

Table 87- SF2, CNQX condition Stim 1 vs. 2 vs. 3 statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (SF 2x) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0.844375	1	0.844375	36.54606	0.000008
Sex	0.101550	1	0.101550	4.39528	0.049658
Treatment	0.003282	1	0.003282	0.14203	0.710443
Sex*Treatment	0.005690	1	0.005690	0.24629	0.625390
Error	0.438984	19	0.023104		
STIM	1.125986	2	0.562993	60.68447	0.000000
STIM*Sex	0.025861	2	0.012931	1.39377	0.260536
STIM*Treatment	0.055969	2	0.027985	3.01643	0.060831
STIM*Sex*Treatment	0.015182	2	0.007591	0.81821	0.448841
Error	0.352541	38	0.009277		

Cell No.	Bonferroni test; variable DV_1 (SF 2x) Probabilities for Post Hoc Tests Error: Between MS = .02310, df = 19.000 Sex	{1} .14945	{2} .07229
1	F		0.048685
2	M	0.048685	

Cell No.	Bonferroni test; variable DV_1 (SF 2x) Probabilities for Post Hoc Tests Error: Within MS = .00928, df = 38.000 STIM	{1} .28685	{2} .06393	{3} -.0131
1	CNQX Stim 1		0.000000	0.000000
2	CNQX Stim 2	0.000000		0.029850
3	CNQX Stim 3	0.000000	0.029850	

Table 88- SF2, MK-801 condition Stim 1 vs. 2 vs. 3 statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (SF 2x) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	1.357218	1	1.357218	61.12401	0.000000
Sex	0.030365	1	0.030365	1.36751	0.255988
Treatment	0.000718	1	0.000718	0.03232	0.859146
Sex*Treatment	0.010857	1	0.010857	0.48895	0.492449
Error	0.444087	20	0.022204		
STIM	0.644919	2	0.322460	16.97289	0.000005
STIM*Sex	0.049114	2	0.024557	1.29257	0.285785
STIM*Treatment	0.012542	2	0.006271	0.33008	0.720803
STIM*Sex*Treatment	0.014560	2	0.007280	0.38318	0.684166
Error	0.759941	40	0.018999		

Cell No.	Bonferroni test, variable DV_1 (SF 2x) Probabilities for Post Hoc Tests Error: Within MS = .01900, df = 40.000 STIM	{1} .26713	{2} .10053	{3} .04422
1	MK-801 Stim 1		0.000452	0.000005
2	MK-801 Stim 2	0.000452		0.494178
3	MK-801 Stim 3	0.000005	0.494178	

Table 89- SF2, CNQX + MK-801 condition Stim 1 vs. 2 vs. 3 statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (SF 2x) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0.785458	1	0.785458	69.76586	0.000000
Sex	0.000470	1	0.000470	0.04178	0.840111
Treatment	0.038990	1	0.038990	3.46318	0.077516
Sex*Treatment	0.000557	1	0.000557	0.04950	0.826193
Error	0.225170	20	0.011258		
STIM	0.921600	2	0.460800	69.82094	0.000000
STIM*Sex	0.001745	2	0.000872	0.13217	0.876576
STIM*Treatment	0.022864	2	0.011432	1.73219	0.189902
STIM*Sex*Treatment	0.024168	2	0.012084	1.83095	0.173440
Error	0.263990	40	0.006600		

Cell No.	Bonferroni test; variable DV_1 (SF 2x) Probabilities for Post Hoc Tests Error: Within MS = .00660, df = 40.000 STIM	{1} .26233	{2} .04795	{3} .00306
1	C+M Stim 1		0.000000	0.000000
2	C+M Stim 2	0.000000		0.188239
3	C+M Stim 3	0.000000	0.188239	

Table 90- SF2, Stim 1 Control vs. CNQX vs. MK-801 vs. CNQX + MK-801 statistics

Parametric repeated measures ANOVA (drug conditions) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (SF 2x) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	7.264681	1	7.264681	378.9737	0.000000
Sex	0.166804	1	0.166804	8.7016	0.008225
Treatment	0.052010	1	0.052010	2.7132	0.115960
Sex*Treatment	0.033300	1	0.033300	1.7371	0.203173
Error	0.364218	19	0.019169		
DRUG	0.051198	3	0.017066	0.9260	0.434150
DRUG*Sex	0.089758	3	0.029919	1.6234	0.193997
DRUG*Treatment	0.030979	3	0.010326	0.5603	0.643403
DRUG*Sex*Treatment	0.005294	3	0.001765	0.0958	0.962077
Error	1.050497	57	0.018430		

Cell No.	Sex	Bonferroni test; variable DV_1 (SF 2x) Probabilities for Post Hoc Tests Error: Between MS = .01917, df = 19.000 {1} .32460	{2} .23527
1	F		0.006010
2	M	0.006010	

Table 91- SF2, Stim 2 Control vs. CNQX vs. MK-801 vs. CNQX + MK-801 statistics

Parametric repeated measures ANOVA (drug conditions) for all sex and housing groups.

Effect	Repeated Measures Analysis of Variance (SF 2x) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0.497770	1	0.497770	28.07118	0.000041
Sex	0.022864	1	0.022864	1.28939	0.270281
Treatment	0.004148	1	0.004148	0.23391	0.634165
Sex*Treatment	0.000043	1	0.000043	0.00243	0.961170
Error	0.336916	19	0.017732		
DRUG	0.035636	3	0.011879	1.42145	0.245922
DRUG*Sex	0.011081	3	0.003694	0.44200	0.723864
DRUG*Treatment	0.044694	3	0.014898	1.78274	0.160712
DRUG*Sex*Treatment	0.002036	3	0.000679	0.08119	0.969986
Error	0.476332	57	0.008357		

Table 92- SF2, Stim 3 Control vs. CNQX vs. MK-801 vs. CNQX + MK-801 statistics

Parametric repeated measures ANOVA (drug conditions) for all sex and housing groups.

Effect	Repeated Measures Analysis of Variance (SF 2x) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0.018669	1	0.018669	1.395961	0.251979
Sex	0.010542	1	0.010542	0.788252	0.385731
Treatment	0.006082	1	0.006082	0.454751	0.508210
Sex*Treatment	0.007705	1	0.007705	0.576105	0.457157
Error	0.254101	19	0.013374		
DRUG	0.041101	3	0.013700	1.624378	0.193779
DRUG*Sex	0.007793	3	0.002598	0.307996	0.819499
DRUG*Treatment	0.036886	3	0.012295	1.457789	0.235686
DRUG*Sex*Treatment	0.013907	3	0.004636	0.549646	0.650431
Error	0.480745	57	0.008434		

A.3.3 SF 3- GLUTAMATE, GABA AND KCL-STIMULATED [^{3}H]NE RELEASE IN HIPPOCAMPUS AND PREFRONTAL CORTEX

Table 93- SF3, Data

Rat #	Sex	Treatment	Sex_treatment	S1 HC Glu	S2 HC GABA	S3 HC KCl	S1 HC GABA	S2 HC Glu	S3 HC KCl	S3 HC KCl average	S1 PFC Glu	S2 PFC GABA	S3 PFC KCl	S1 PFC GABA	S2 PFC GLU	S3 PFC KCl	S3 PFC KCl average
101	M	S	MS	0.1781	0.1840	3.3824	-0.0966	1.2122	6.0904	4.7364	0.1471	0.1995	9.4818	-0.0409	0.1588	6.2288	7.8553
106	M	I	MI	0.0937	0.1135	6.0671	-0.1144	-0.1494	4.6257	5.3464	-0.0092	0.0908	5.0905	-0.0822	0.1391	6.8752	5.9829
107	M	I	MI	0.1924	0.1599	6.1064	0.0780	0.0656	3.7640	4.9352	0.1664	0.0400	4.1226	0.1170	-0.3349	5.8208	4.9717
102	M	S	MS	0.0970	0.0915	2.9529	0.2274	-0.0191	8.4807	5.7168	0.1790	0.1053	6.6462	0.1187	0.2195	8.0259	7.3360
103	M	S	MS	0.3927	0.0763	7.1714	0.5736	0.3220	5.0620	6.1167	0.4031	0.1481	8.0861	-0.0492	0.0491	4.1647	6.1254
108	M	I	MI	0.1569	0.2187	4.6172	0.2201	0.3342	7.1308	5.8740	0.2162	0.1161	7.1776	0.2441	0.2632	6.7191	6.9484
111	F	S	FS	0.3016	0.2502	8.0706	0.1416	0.0956	4.5171	6.2938	0.0466	0.0650	4.6989	0.0973	0.0785	4.7051	4.7020
116	F	I	FI	0.0381	0.2381	4.8324	-0.1461	-0.0784	3.3304	4.0814	0.0036	-0.0814	3.9120	0.0321	0.0843	4.4655	4.1888
117	F	I	FI	0.0334	0.0759	4.5666	0.0461	0.0207	4.4702	4.5184	-0.0378	-0.0173	2.5906	0.0000	0.0588	6.3690	4.4798
112	F	S	FS	0.1035	0.0741	5.5020	0.1685	0.1332	5.5622	5.5321	0.0000	0.0131	7.3189	0.0934	0.0149	7.9666	7.6427
113	F	S	FS	0.1833	0.1844	1.9461	0.0361	-0.0305	2.4244	2.1852	0.0398	-0.0804	2.1372	0.0048	-0.0090	4.3588	3.2480
118	F	I	FI	0.0867	0.0872	2.2025	0.2886	-0.0294	2.6486	2.4256	0.2619	0.0138	4.6605	0.2053	0.0058	4.5278	4.5941
119	F	I	FI	0.1006	0.0361	4.5018	0.1369	0.1642	4.7232	4.6125	0.1467	0.0529	5.9920	0.0810	0.0528	5.3385	5.6653
114	F	S	FS	0.2474	0.1469	6.0522	0.2322	0.2477	6.6624	6.3573	0.0881	0.1254	3.7635	0.2402	0.1752	5.8622	4.8129
125	F	I	FI	0.3052	0.1154	3.5267	-0.0017	0.0524	1.4587	2.4927	0.2367	0.1691	4.7612	0.0904	0.2750	5.1978	4.9795
121	F	S	FS	0.0077	0.0365	1.5963	0.0642	-0.3334	2.4550	2.0257	0.0782	0.0943	5.0043	0.1735	0.1998	5.1899	5.0971
122	F	S	FS	0.1500	0.0118	3.3866	0.2058	0.2022	4.7339	4.0603	0.0840	-0.0114	5.5653	0.1189	0.3652	7.8195	6.6924
126	F	I	FI	0.1180	0.1766	6.0621	0.3289	0.0891	5.5065	5.7843	0.1548	0.0907	9.3382	0.1810	0.1114	7.5167	8.4274
127	F	I	FI	0.1411	0.1158	2.3821				2.3821	0.2233	0.1926	5.4499	0.0987	0.0640	2.9305	4.1902
123	F	S	FS	0.0155	0.1496	4.6194				4.6194	0.3126	0.1490	7.4382	-0.1226	-0.0160	4.3890	5.9136
124	F	S	FS	0.2598	0.1394	5.1174	0.0738	0.1217	2.9628	4.0401	0.1287	0.1982	5.7977	0.1520	0.2740	6.9316	6.3647
128	F	I	FI	0.4155	0.1171	7.5202	0.0255	0.2440	3.8980	5.7091	0.1635	1.1270	7.1274	0.2429	0.0890	6.4676	6.7975
133	F	I	FI	0.3694	0.1202	2.1622	0.1986	0.2111	2.8389	2.5005	0.2248	-0.0023	2.2632	0.1244	0.2075	2.4969	2.3801
129	F	S	FS	0.3571	0.2406	3.9059	0.0791	0.1190	6.5099	5.2079	0.2082	0.2315	4.7332	0.0684	0.0382	1.5795	3.1564
130	F	S	FS	0.4340	0.2926	5.0725	0.1582	0.1770	2.6930	3.8827	0.1361	0.1466	5.4854	0.2819	0.2410	6.6550	6.0702
134	F	I	FI	0.4003	0.1900	4.8751	0.0461	0.1389	3.9307	4.4029	0.1825	0.0174	4.9753	0.0665	0.0828	3.3574	4.1663
135	F	I	FI	0.1990	0.2264	6.4158	0.0985	0.2325	4.9673	5.6916	0.3554	0.2594	5.9141	0.0792	0.0525	3.9479	4.9310
131	F	S	FS	0.2184	0.2156	6.2326	0.2561	0.1757	7.5612	6.8969	0.1229	0.1168	5.1640	0.0409	0.0521	4.8895	5.0268
132	F	S	FS	0.1372	0.0733	3.1738	0.2340	0.1493	3.3981	3.2860	0.1511	0.1530	5.2591	0.4176	0.1296	4.4969	4.8780
136	F	I	FI	0.2545	0.2554	4.4278	-0.3798	-0.3258	7.3207	5.8743	0.1029	0.0350	3.8385	0.0365	0.0893	4.9235	4.3810
137	M	S	MS	0.6676	0.2610	6.7002	0.1145	0.1359	5.0520	5.8761	0.2654	-0.0110	5.6417	-0.1026	0.0985	8.5824	7.1120
140	M	I	MI	0.3130	0.1641	8.2287	-0.0239	0.0485	5.9791	7.1039	0.1318	0.5407	5.6959	0.1254	-0.1164	6.2218	5.9589
141	M	I	MI	0.2869	-0.1564	5.5876	0.0961	0.1846	4.9673	5.2775	-0.0075	0.0241	4.7114	0.0617	0.1189	4.0856	4.3985
138	M	S	MS								0.1749	-0.0208	4.3134	0.0682	0.2332	6.0311	5.1722
139	M	S	MS	0.4302	-0.0116	3.2780	0.0368	-0.0262	3.7908	3.5344	0.1863	0.2335	7.4264	-0.0932	0.7022	5.0988	6.2626
142	M	I	MI	0.1797	0.0399	3.6222	0.0108	0.4868	4.5771	4.0997	0.2336	0.8684	6.6818	-0.0487	-0.3991	6.0687	6.3752

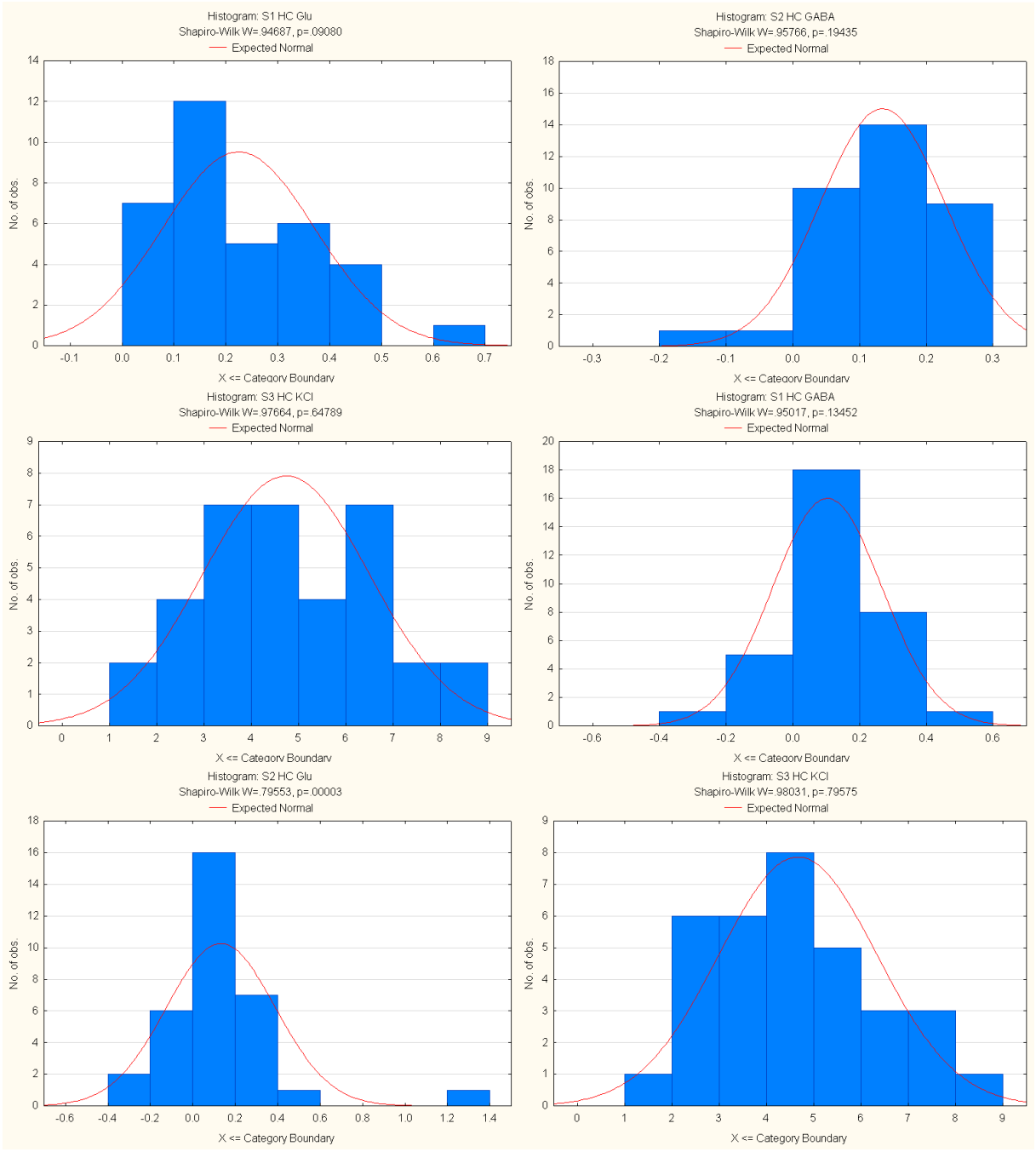


Figure 90- SF3, Histograms 1/3

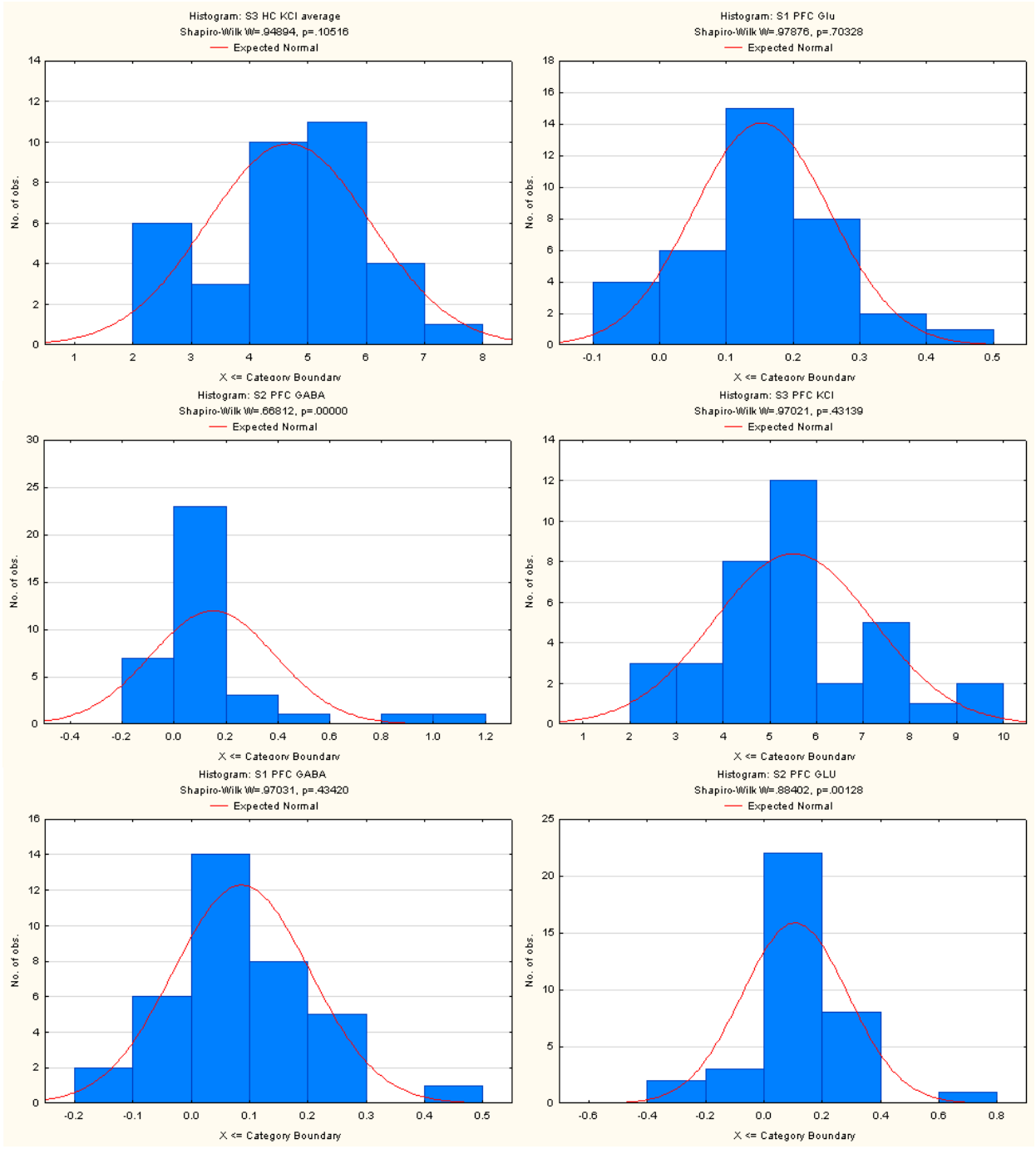


Figure 91- SF3, Histograms 2/3

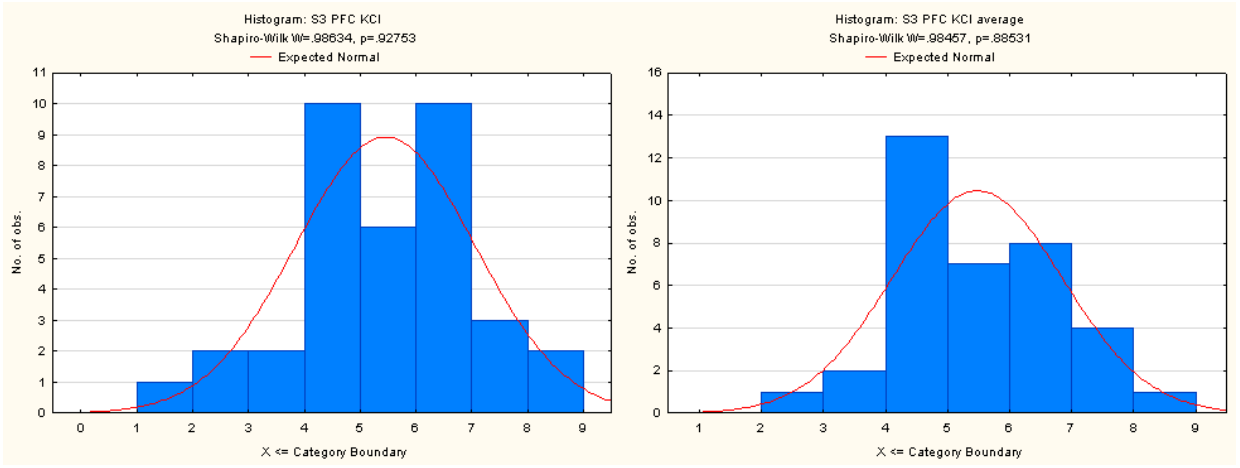


Figure 92- SF3, Histograms 3/3

Table 94- SF3, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
S1 HC Glu	M	S	5	0.353113	0.097025	0.667584	0.225057
S2 HC GABA	M	S	5	0.120233	-0.011559	0.260969	0.104876
S3 HC KCl	M	S	5	4.696970	2.952903	7.171404	2.056653
S1 HC GABA	M	S	5	0.171142	-0.096646	0.573595	0.254017
S2 HC Glu	M	S	5	0.324957	-0.026183	1.212224	0.515918
S3 HC KCl	M	S	5	5.695167	3.790791	8.480655	1.757585
S3 HC KCl average	M	S	5	5.196069	3.534371	6.116708	1.066966
S1 PFC Glu	M	S	6	0.225966	0.147069	0.403084	0.095436
S2 PFC GABA	M	S	6	0.109096	-0.020772	0.233514	0.106292
S3 PFC KCl	M	S	6	6.932592	4.313360	9.481806	1.826560
S1 PFC GABA	M	S	6	-0.016508	-0.102600	0.118651	0.089876
S2 PFC GLU	M	S	6	0.243569	0.049118	0.702211	0.235405
S3 PFC KCl	M	S	6	6.355270	4.164679	8.582365	1.688147
S3 PFC KCl average	M	S	6	6.643931	5.172230	7.855301	0.974243
S1 HC Glu	M	I	6	0.203764	0.093703	0.312956	0.082291
S2 HC GABA	M	I	6	0.089946	-0.156383	0.218747	0.134692
S3 HC KCl	M	I	6	5.704869	3.622234	8.228703	1.561817
S1 HC GABA	M	I	6	0.044450	-0.114419	0.220099	0.114622
S2 HC Glu	M	I	6	0.161714	-0.149402	0.486803	0.225749
S3 HC KCl	M	I	6	5.173998	3.763975	7.130823	1.197084
S3 HC KCl average	M	I	6	5.439433	4.099679	7.103880	1.004323
S1 PFC Glu	M	I	6	0.121881	-0.009175	0.233610	0.107128
S2 PFC GABA	M	I	6	0.280035	0.024116	0.868433	0.346391
S3 PFC KCl	M	I	6	5.579975	4.122640	7.177616	1.174533
S1 PFC GABA	M	I	6	0.069547	-0.082204	0.244114	0.120765
S2 PFC GLU	M	I	6	-0.054877	-0.399102	0.263161	0.271905
S3 PFC KCl	M	I	6	5.965218	4.085566	6.875227	1.002696
S3 PFC KCl average	M	I	6	5.772596	4.398480	6.948368	0.933180
S1 HC Glu	F	S	12	0.201297	0.007654	0.434036	0.128970
S2 HC GABA	F	S	12	0.151257	0.011761	0.292607	0.089338
S3 HC KCl	F	S	12	4.556293	1.596308	8.070590	1.862423
S1 HC GABA	F	S	11	0.149973	0.036128	0.256057	0.077346
S2 HC Glu	F	S	11	0.096160	-0.333368	0.247739	0.159061
S3 HC KCl	F	S	11	4.498190	2.424370	7.561238	1.860190
S3 HC KCl average	F	S	12	4.532294	2.025668	6.896933	1.587482
S1 PFC Glu	F	S	12	0.116347	0.000000	0.312552	0.083281
S2 PFC GABA	F	S	12	0.100106	-0.080352	0.231498	0.089941
S3 PFC KCl	F	S	12	5.197145	2.137184	7.438206	1.415101
S1 PFC GABA	F	S	12	0.130531	-0.122619	0.417616	0.139499
S2 PFC GLU	F	S	12	0.128629	-0.015970	0.365214	0.122953
S3 PFC KCl	F	S	12	5.403643	1.579512	7.966603	1.780737
S3 PFC KCl average	F	S	12	5.300394	3.156368	7.642736	1.322018
S1 HC Glu	F	I	12	0.205137	0.033442	0.415516	0.140092
S2 HC GABA	F	I	12	0.146196	0.036123	0.255369	0.069781
S3 HC KCl	F	I	12	4.456268	2.162154	7.520193	1.696163
S1 HC GABA	F	I	11	0.058326	-0.379823	0.328855	0.198098
S2 HC Glu	F	I	11	0.065386	-0.325757	0.244020	0.167815
S3 HC KCl	F	I	11	4.099380	1.458696	7.320747	1.580007
S3 HC KCl average	F	I	12	4.206271	2.382098	5.874271	1.426949
S1 PFC Glu	F	I	12	0.168192	-0.037782	0.355423	0.108476
S2 PFC GABA	F	I	12	0.154738	-0.081429	1.127048	0.321297
S3 PFC KCl	F	I	12	5.068573	2.263216	9.338185	1.937951
S1 PFC GABA	F	I	12	0.103183	-0.000002	0.242942	0.073364
S2 PFC GLU	F	I	12	0.097768	0.005813	0.275045	0.073486
S3 PFC KCl	F	I	12	4.794919	2.496909	7.516705	1.503900
S3 PFC KCl average	F	I	12	4.931746	2.380062	8.427445	1.509417

Table 95- SF3, HC Glu Stim 1 vs. GABA Stim 2 vs. GABA Stim 1 vs. Glu Stim 2 statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (4.3 SF 3) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	3.007428	1	3.007428	86.36654	0.000000
Sex	0.062385	1	0.062385	1.79156	0.191136
Treatment	0.165003	1	0.165003	4.73852	0.037779
Sex*Treatment	0.051909	1	0.051909	1.49072	0.231939
Error	1.009829	29	0.034822		
STIM	0.334088	3	0.111363	3.89321	0.011610
STIM*Sex	0.175011	3	0.058337	2.03944	0.114247
STIM*Treatment	0.037192	3	0.012397	0.43341	0.729611
STIM*Sex*Treatment	0.020564	3	0.006855	0.23964	0.868477
Error	2.488578	87	0.028604		

Cell No.	Bonferroni test; variable DV_1 (4.3 SF 3) Probabilities for Post Hoc Tests Error: Between MS = .03482, df = 29.000 Treatment	{1} .18157	{2} .12234
1	S		0.078722
2	I	0.078722	

Cell No.	Bonferroni test; variable DV_1 (4.3 SF 3) Probabilities for Post Hoc Tests Error: Within MS = .02860, df = 87.000 STIM	{1} .23360	{2} .13469	{3} .10345	{4} .13249
1	HC S1 glu		0.118345	0.014457	0.103338
2	HC S2 gaba	0.118345		1.000000	1.000000
3	HC S1 gaba	0.014457	1.000000		1.000000
4	HC S2 glu	0.103338	1.000000	1.000000	

Table 96- SF3, PFC Glu Stim 1 vs. GABA Stim 2 vs. GABA Stim 1 vs. Glu Stim 2 statistics

Parametric repeated measures ANOVA (stimulations) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (4.3 SF 3) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	1.956645	1	1.956645	66.60789	0.000000
Sex	0.000216	1	0.000216	0.00735	0.932195
Treatment	0.004731	1	0.004731	0.16104	0.690872
Sex*Treatment	0.018780	1	0.018780	0.63931	0.429855
Error	0.940018	32	0.029376		
STIM	0.181642	3	0.060547	2.31704	0.080464
STIM*Sex	0.111996	3	0.037332	1.42864	0.239198
STIM*Treatment	0.326270	3	0.108757	4.16194	0.008107
STIM*Sex*Treatment	0.225827	3	0.075276	2.88067	0.039886
Error	2.508602	96	0.026131		

Bonferroni test; variable DV_1 (4.3 SF 3)
Probabilities for Post Hoc Tests
Error: Between; Within; Pooled MS = .02694, df = 127.65

Cell No.	Treatment	STIM	{1} .15289	{2} .10310	{3} .08152	{4} .16694	{5} .15276	{6} .19650	{7} .09197	{8} .04689
1	S	PFC S1 glu		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
2	S	PFC S2 gaba	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
3	S	PFC S1 gaba	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000
4	S	PFC S2 glu	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	0.840755
5	I	PFC S1 glu	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
6	I	PFC S2 gaba	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	0.184912
7	I	PFC S1 gaba	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000
8	I	PFC S2 glu	1.000000	1.000000	1.000000	0.840755	1.000000	0.184912	1.000000	

Bonferroni test; variable DV_1 (4.3 SF 3)
Probabilities for Post Hoc Tests
Error: Between; Within; Pooled MS = .02694, df = 127.65

Cell No.	Sex	Treatment	STIM	{1} .22597	{2} .10910	{3} -.0165	{4} .24357	{5} .12188	{6} .28003	{7} .06955	{8} -.0549	{9} .11635	{10} .10011	{11} .13053	{12} .12863	{13} .16819	{14} .15474	{15} .10318	{16} .09777
1	M	S	PFC S1 glu		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.435544	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
2	M	S	PFC S2 gaba	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
3	M	S	PFC S1 gaba	1.000000	1.000000		0.770260	1.000000	0.260701	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
4	M	S	PFC S2 glu	1.000000	1.000000	0.770260		1.000000	1.000000	1.000000	0.244652	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
5	M	I	PFC S1 glu	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
6	M	I	PFC S2 gaba	1.000000	1.000000	0.260701	1.000000	1.000000		1.000000	0.063104	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
7	M	I	PFC S1 gaba	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
8	M	I	PFC S2 glu	0.435544	1.000000	1.000000	0.244652	1.000000	0.063104	1.000000		1.000000	1.000000	1.000000	1.000000	0.897665	1.000000	1.000000	1.000000
9	F	S	PFC S1 glu	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
10	F	S	PFC S2 gaba	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000	1.000000
11	F	S	PFC S1 gaba	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000	1.000000
12	F	S	PFC S2 glu	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000	1.000000
13	F	I	PFC S1 glu	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	0.897665	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000	1.000000
14	F	I	PFC S2 gaba	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000	1.000000
15	F	I	PFC S1 gaba	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000		1.000000
16	F	I	PFC S2 glu	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	1.000000	

Table 97- SF3, Glu Stim 1 PFC vs. HC statistics

Parametric repeated measures ANOVA (brain areas) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (4.3 SF 3) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	2.417785	1	2.417785	131.5985	0.000000
Sex	0.047027	1	0.047027	2.5597	0.119765
Treatment	0.040548	1	0.040548	2.2070	0.147487
Sex*Treatment	0.095604	1	0.095604	5.2037	0.029565
Error	0.569545	31	0.018372		
AREA	0.096423	1	0.096423	8.0507	0.007950
AREA*Sex	0.005546	1	0.005546	0.4631	0.501247
AREA*Treatment	0.006466	1	0.006466	0.5399	0.468005
AREA*Sex*Treatment	0.000157	1	0.000157	0.0131	0.909445
Error	0.371287	31	0.011977		

Cell No.	Bonferroni test; variable DV_1 (4.3 SF 3) Probabilities for Post Hoc Tests Error: Between MS = .01837, df = 31.000 Sex	Treatment	{1} .29465	{2} .16282	{3} .15882	{4} .18666
1	M	S		0.181283	0.073136	0.254522
2	M	I	0.181283		1.000000	1.000000
3	F	S	0.073136	1.000000		1.000000
4	F	I	0.254522	1.000000	1.000000	

Cell No.	Bonferroni test; variable DV_1 (4.3 SF 3) Probabilities for Post Hoc Tests Error: Within MS = .01198, df = 31.000 AREA	{1} .22472	{2} .15219
1	HC S1 glu		0.009326
2	PFC S1 glu	0.009326	

Table 98- SF3, GABA Stim 1 PFC vs. HC statistics

Parametric repeated measures ANOVA (brain areas) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (4.3 SF 3) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	0.468811	1	0.468811	18.42186	0.000180
Sex	0.041645	1	0.041645	1.63645	0.210955
Treatment	0.024910	1	0.024910	0.97885	0.330667
Sex*Treatment	0.012672	1	0.012672	0.49795	0.486033
Error	0.738009	29	0.025449		
AREA	0.015562	1	0.015562	1.30403	0.262820
AREA*Sex	0.047523	1	0.047523	3.98236	0.055448
AREA*Treatment	0.067134	1	0.067134	5.62572	0.024553
AREA*Sex*Treatment	0.032217	1	0.032217	2.69971	0.111167
Error	0.346070	29	0.011933		

Cell No.	Bonferroni test; variable DV_1 (4.3 SF 3) Probabilities for Post Hoc Tests Error: Between; Within; Pooled MS = .01869, df = 51.295 Treatment	AREA	{1} .15659	{2} .09511	{3} .05343	{4} .09157
1	S	HC S1 gaba		0.733680	0.209739	1.000000
2	S	PFC S1 gaba	0.733680		1.000000	1.000000
3	I	HC S1 gaba	0.209739	1.000000		1.000000
4	I	PFC S1 gaba	1.000000	1.000000	1.000000	

Table 99 SF3, HC KCl Stim 3 (after Glu, GABA) statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for (Glu, GABA) S3 HC KCl (4.3 SF 3) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	706.7230	1	706.7230	221.4825	0.000000
Sex	3.6189	1	3.6189	1.1341	0.295117
Treatment	1.5454	1	1.5454	0.4843	0.491652
Sex*Treatment	2.3016	1	2.3016	0.7213	0.402232
Error	98.9171	31	3.1909		

Table 100 SF3, HC KCl Stim 3 (after GABA, Glu) statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for (GABA, Glu) S3 HC KCl (4.3 SF 3) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	690.9102	1	690.9102	253.3407	0.000000
Sex	9.4080	1	9.4080	3.4497	0.073446
Treatment	1.5431	1	1.5431	0.5658	0.457989
Sex*Treatment	0.0273	1	0.0273	0.0100	0.920996
Error	79.0887	29	2.7272		

Table 101 SF3, PFC KCl Stim 3 (after Glu, GABA) statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for (Glu, GABA) S3 PFC KCl (4.3 SF 3) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	1037.700	1	1037.700	382.0385	0.000000
Sex	10.097	1	10.097	3.7172	0.062769
Treatment	4.388	1	4.388	1.6154	0.212893
Sex*Treatment	2.997	1	2.997	1.1032	0.301430
Error	86.919	32	2.716		

Table 102 SF3, PFC KCl Stim 3 (after GABA, Glu) statistics

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for (GABA, Glu) S3 PFC KCl (4.3 SF 3) Sigma-restricted parameterization Effective hypothesis decomposition				
	SS	Degr. of Freedom	MS	F	p
Intercept	1014.215	1	1014.215	410.6326	0.000000
Sex	9.005	1	9.005	3.6460	0.065205
Treatment	1.995	1	1.995	0.8078	0.375492
Sex*Treatment	0.096	1	0.096	0.0387	0.845248
Error	79.036	32	2.470		

Table 103- SF3, KCl Stim 3 HC vs. PFC statistics

Parametric repeated measures ANOVA (brain areas) for all sex and housing groups. Followed by Bonferroni post hoc.

Effect	Repeated Measures Analysis of Variance (Spreadsheet14) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	1678.814	1	1678.814	718.8032	0.000000
Sex	17.950	1	17.950	7.6854	0.009333
Treatment	2.451	1	2.451	1.0495	0.313547
Sex*Treatment	0.049	1	0.049	0.0208	0.886257
Error	72.403	31	2.336		
AREA	11.941	1	11.941	9.7157	0.003923
AREA*Sex	0.317	1	0.317	0.2582	0.614968
AREA*Treatment	1.976	1	1.976	1.6074	0.214295
AREA*Sex*Treatment	1.750	1	1.750	1.4242	0.241772
Error	38.101	31	1.229		

Cell No.	Bonferroni test; variable DV_1 (4.3 SF 3) Probabilities for Post Hoc Tests Error: Between MS = 2.3356, df = 31.000 Sex	{1} 5.8156	{2} 4.7427
1	M		0.010427
2	F	0.010427	

Cell No.	Bonferroni test; variable DV_1 (4.3 SF 3) Probabilities for Post Hoc Tests Error: Within MS = 1.2291, df = 31.000 AREA	{1} 4.6708	{2} 5.4889
1	S3 HC KCl average		0.004237
2	S3 PFC KCl average	0.004237	

A.4 ELISA AND BCA ASSAY

Instructions for use

Noradrenaline Research ELISA ™

REF **BA E-5200**

96

RUO

Noradrenaline Research ELISA

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of Noradrenaline (Norepinephrine).
Flexible test system for various biological sample types and volumes.
Noradrenaline (Norepinephrine) is extracted by using a cis-diol-specific affinity gel, acylated and then derivatized enzymatically.
The competitive ELISA kit uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The derivatized standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. After the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standard concentrations.

2. Advice on handling the test

2.1 Reliability of the test results
In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RILIBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test: the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.
It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.
The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

2.2 Complaints
In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty
This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal
Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference
Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions
Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Storage and stability
Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4.1 Contents of the kit

BA D-0032	96 st	**Microtiter Plate**	1 x 96 wells	12 strips, 8 wells each, break apart
BA D-0090	FOILS	**Adhesive Foil**	1 x 4	ready for use
BA E-0030	WASH-CONC 50X	**Wash Buffer Concentrate**	1 x 20 mL	Concentrate. Dilute content with dist. water to a final volume of 1000 mL
BA E-0040	CONJUGATE	**Enzyme Conjugate**	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of TMB
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4
BA E-0231	96 NAD NMN	**Noradrenaline-Normetanephrine Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, pre-coated, yellow coloured
BA E-5210	NAD-AS	**Noradrenaline Antiserum**	1 x 6 mL	from rabbit, ready for use, yellow coloured, yellow screw cap
BA R-0050	ADJUST-BUFF	**Adjustment Buffer**	1 x 4 mL	ready for use
BA R-4617	TE-BUFF	**TE Buffer**	1 x 4 mL	ready for use
BA R-5601	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA R-5602	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA R-5603	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA R-5604	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA R-5605	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA R-5606	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA R-5651	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA R-5652	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA R-6611	ACYL-BUFF	**Acylation Buffer**	1 x 20 mL	ready for use
BA R-6612	ACYL-REAG	**Acylation Reagent**	1 x 3 mL	ready for use
BA R-6614	COENZYME	**Coenzyme**	1 x 4 mL	ready for use, S-adenosyl-L-methionine
BA R-6615	ENZYME	**Enzyme**	4 x 1 mL	lyophilized, contains the enzyme COMT
BA R-6618	EXTRACT-PLATE 48	**Extraction Plate**	2 x 48 wells	coated with boronate affinity gel
BA R-6619	HCL	**Hydrochloric Acid**	1 x 20 mL	ready for use, yellow coloured, contains 0.025 M HCl

4.2 Additional materials and equipment required but not provided with the kit
- Calibrated variable precision micropipettes (e.g. 1-10 µL / 10-100 µL / 100-1000 µL)
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm (reference filter 620 – 650 nm)
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

5. Sample collection and storage
Storage: up to 6 hours at 2 – 8 °C; for longer periods (up to 6 months) at - 20°C or – 80 °C.
Advice for the preservation of the biological sample: to prevent catecholamine degradation add EDTA (final concentration 1mM) and sodium metabisulfite (final concentration 4 mM) to the sample.

6. Test procedure
Allow reagents and samples to reach room temperature. Duplicate determinations are recommended.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 2-8°C

Enzyme Solution
Reconstitute the content of the vial labelled 'Enzyme' with 1 mL distilled water and mix thoroughly. Add 0.3 mL of Coenzyme followed by 0.7 mL of Adjustment Buffer. The total volume of the Enzyme Solution is 2.0 mL.
⚠ *The Enzyme Solution has to be prepared freshly prior to the assay (not longer than 10 - 15 minutes in advance). Discard after use!*

6.2 Sample preparation
The Noradrenaline Research ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to fit the protocol to his specific needs.
- Avoid excess of acid: excess of acid might exceed the buffer capacity of the extraction buffer. A pH > 7.0 during the extraction is mandatory.
- Prevent catecholamine degradation by adding preservatives to the sample (see 5. Sample collection and Storage).
- Avoid chaotropic chemicals like perchloric acid. The high salt content might reduce the recovery of Noradrenaline. If your samples already contain high amounts of perchloric acid, neutralize the sample prior to the extraction step.
- Tissue samples can be homogenised in 0.01 N HCl in the presence of EDTA and sodium metabisulfite. Under these conditions Noradrenaline is positively charged which reduces binding to proteins and optimizes solubility.
- Avoid samples that contain substances with a cis-diol structure. These will reduce the recovery of the Noradrenaline.
- It is advisable to perform a "Proof of Principle" to determine the recovery of the Noradrenaline in your samples. Prepare a stock solution of Noradrenaline. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.
- The used sample volume determines the sensitivity of this test. Determine the sample volume needed to determine the Noradrenaline in your sample by testing different amounts of sample volume.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

Figure 93- NE ELISA instruction manual 1/2

6.3 Extraction and acylation

The Research ELISA offers a flexible test system for various biological sample types and sizes. Step 1 of the extraction procedure depends on the sample volume:

- in case you have sample volumes between 1 – 100 µL follow **1.1**
- in case you have sample volumes between 100 – 500 µL follow **1.2**
- in case you have sample volumes between 500 – 750 µL follow **1.3**

⚠ ***Within a run it is only possible to measure samples with the same volume!***

	1.1	1.2	1.3
1.	**Sample volume 1 – 100 µL** Pipette into the respective wells of the Extraction Plate: **10 µL standards, 10 µL controls and 1 – 100 µL of the sample.** Fill up each well with distilled water to a **final volume** of 100 µl (e.g. 10 µl standard plus 90 µl dist. water).	**Sample volume 100 – 500 µL** Pipette into the respective wells of the Extraction Plate: **10 µL standards, 10 µL controls and 100 – 500 µL of the sample.** Fill up each well with distilled water to a **final volume** of 500 µl (e.g. 10 µl standard plus 490 µl dist. water).	**Sample volume 500 – 750 µL** Pipette into the respective wells of the Extraction Plate: **10 µL of Standards, 10 µL of controls and 500 – 750 µL of sample.** Fill up each well with distilled water to a **final volume** of 750 µl (e.g. 10 µl standard plus 740 µl dist. water).
2.	Pipette **25 µL** of **TE Buffer** into all wells		
3.	Cover the plate with adhesive foil. Shake **60 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).		
4.	Remove the foil and empty the plate. Blot dry by tapping the inverted plate on absorbent material.		
5.	Pipette **1 mL** of **Wash Buffer** into all wells.		
6.	Shake **5 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).		
7.	Blot dry by tapping the inverted plate on absorbent material.		
8.	**Wash one more time** as described (step 5, 6 and 7)!		
9.	Pipette **150 µL** of **Acylation Buffer** into all wells.		
10.	Pipette **25 µL** of **Acylation Reagent** into all wells.		
11.	Shake **20 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).		
12.	Empty the plate and blot dry by tapping the inverted plate on absorbent material.		
13.	Pipette **1 mL** of **Wash Buffer** into all wells.		
14.	Shake **5 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).		
15.	Blot dry by tapping the inverted plate on absorbent material.		
16.	**Wash one more time** as described (step 13, 14, 15).		
17.	Pipette **100 µL** of **Hydrochloric Acid** into all wells.		
18.	Cover plate with adhesive foil. Shake **10 min** at **RT** (20-25°C) on an o shaker (approx. 600 rpm). ⚠ ***Do not decant the supernatant thereafter!*** **90 µL of the supernatant is needed for the subsequent enzymatic conversion**		

6.4 Enzymatic Conversion

1.	Pipette **90 µL** of the **extracted standards, controls** and **samples** into the respective wells of the **Microtiter Plate.**
2.	Add **25 µL** of **Enzyme Solution** (refer to 6.1) to all wells.
3.	Cover plate with **Adhesive Foil.** Shake **1 min** at **RT (20-25°C)** on a shaker to mix.
4.	Incubate for **2 hours** at **37°C.** The following volumes of the supernatants are needed for the subsequent ELISA: **Noradrenaline** **100 µL**

6.5 Noradrenaline ELISA

1.	Pipette **100 µL** of **standards, controls** and **samples** from the **Enzyme Plate** (refer to 6.4) into the respective pre-coated **Noradrenaline Microtiter Strips.**
2.	Pipette **50 µL** of the respective **Noradrenaline Antiserum** into all wells.
3.	Cover the plate with **Adhesive Foil.** Incubate for **1 min** at **RT** (20-25°C) on a **shaker.**
4.	Incubate for **15 – 20 hours** (overnight) at **2 – 8 °C.**
5.	Remove the foil and discard or aspirate the contents of the wells and **wash** each well **4 times** thoroughly with 300 µL **Wash Buffer.** Blot dry by tapping the inverted plate on absorbent material.
6.	Pipette **100 µL** of **Enzyme Conjugate** into all wells.
7.	Cover the plate with **Adhesive Foil** and incubate **30 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm).
8.	Remove the foil and discard or aspirate the contents of the wells and **wash** each well **4 times** thoroughly with 300 µl **Wash Buffer.** Blot dry by tapping the inverted plate on absorbent material.
9.	Pipette **100 µL** of **Substrate** into all wells.
10.	Incubate **20-30 min** at **RT** (20-25°C) on a **shaker** (approx. 600 rpm). ⚠ ***Avoid exposure to direct sun light!***
11.	Pipette **100 µL** of **Stop Solution** into all wells.
12.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

The calibration curve from which the concentrations in the samples can be read off, is obtained by plotting the absorbance readings (calculate the mean absorbance) measured for the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
The use of a non-linear regression for curve fitting (e.g. spline, 4- parameter, akima) is recommended.

The standards refer to:

	Concentration of the standards (ng/mL)					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Noradrenaline	0	0.2	0.6	2	8	32

⚠ **The concentrations of the samples taken from the standard curve have to be multiplied by a correction factor.**

$$\text{Correction factor} = \frac{\textbf{10 µL (volume of standards extracted)}}{\textbf{sample volume (µL) extracted}}$$

Example: 750µL of the sample is extracted and the concentration taken from the standard curve is 0.15 ng/mL Noradrenaline.
Correction factor = 10/750 = 0.013
Concentration of the sample = 0.15 ng/mL x 0.013 = 0.002 ng/mL = 2 pg/mL Noradrenaline

7.1 Quality control

It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

7.2 Calibration

The binding of the antisera and the enzyme conjugates and the activity of the enzyme used are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. The extinction values also depend on the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

⚠ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*

8. Assay characteristics

	Substance	Cross Reactivity (%)
		Noradrenaline
Analytical Specificity (Cross Reactivity)	Derivatized Adrenaline	0.14
	Derivatized Noradrenaline	100
	Derivatized Dopamine	0.2
	Metanephrine	< 0.003
	Normetanephrine	0.48
	3-Methoxytyramine	< 0.003
	3-Methoxy-4-hydroxyphenylglycol	0.01
	Tyramine	< 0.003
	Phenylalanine, Caffeinic acid, L-Dopa, Homovanillic acid, Tyrosine, 3-Methoxy-4-hydroxymandelic acid	< 0.003

Sensitivity (Limit of Detection)	**Noradrenaline**
	0.1 ng/mL x C*

C* = Correction factor (refer to 7.)

Analytical Sensitivity (750 µl undiluted sample)	**Noradrenaline**
	1.3 pg/mL

Functional Sensitivity (750 µl undiluted sample)	**Noradrenaline**
	2 pg/mL

Precision				
Intra-Assay Human EDTA-Plasma				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Noradrenaline	high	1377.4 ± 403.6	161.2	11.7
	medium	502.6 ± 126.9	42.3	8.4
	low	32.7 ± 15.3	5.1	15.6
Intra-Assay Cell Culture Medium (RPMI)				
	Sample	Mean ± 3 SD (pg/mL)	SD (pg/mL)	CV (%)
Noradrenaline	high	2027.8 ± 712.5	237.5	11.7
	medium	716.5 ± 179.7	59.9	8.4
	low	46.0 ± 16.8	5.6	12.2

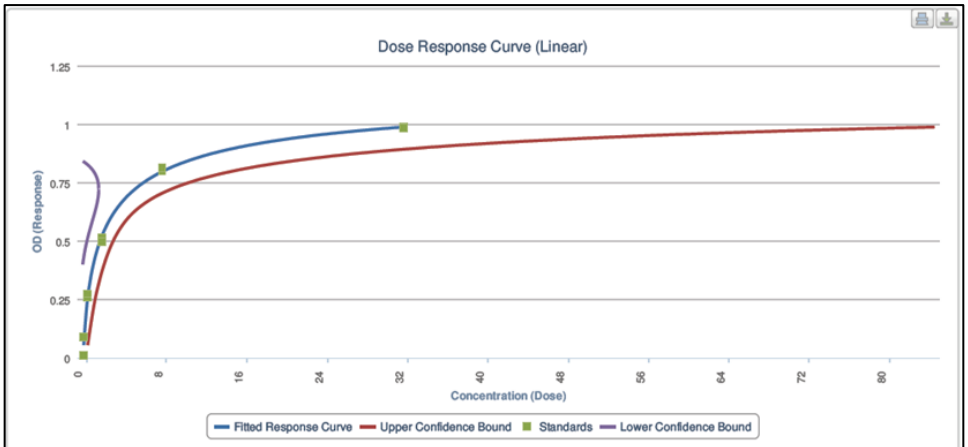
Recovery	Mean (%)	Range (%)	SD (%)	CV (%)
Noradrenaline				
Human EDTA-Plasma	116.5	104.8 – 125.6	8.0	6.9
Cell Culture Medium	96.7	70.6 – 124.7	17.1	17.7

⚠ ***For current literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!

Figure 94- NE ELISA instruction manual 2/2



Regression Formula: $y = d + \frac{a-d}{1+(x/c)^b}$

Inverse Formula: $x = c\left(\frac{a-d}{y-d} - 1\right)^{\frac{1}{b}}$

Where:
y = Response Value eg. OD
x = Dose Value eg. Concentration
and the constants/parameters are as follows:

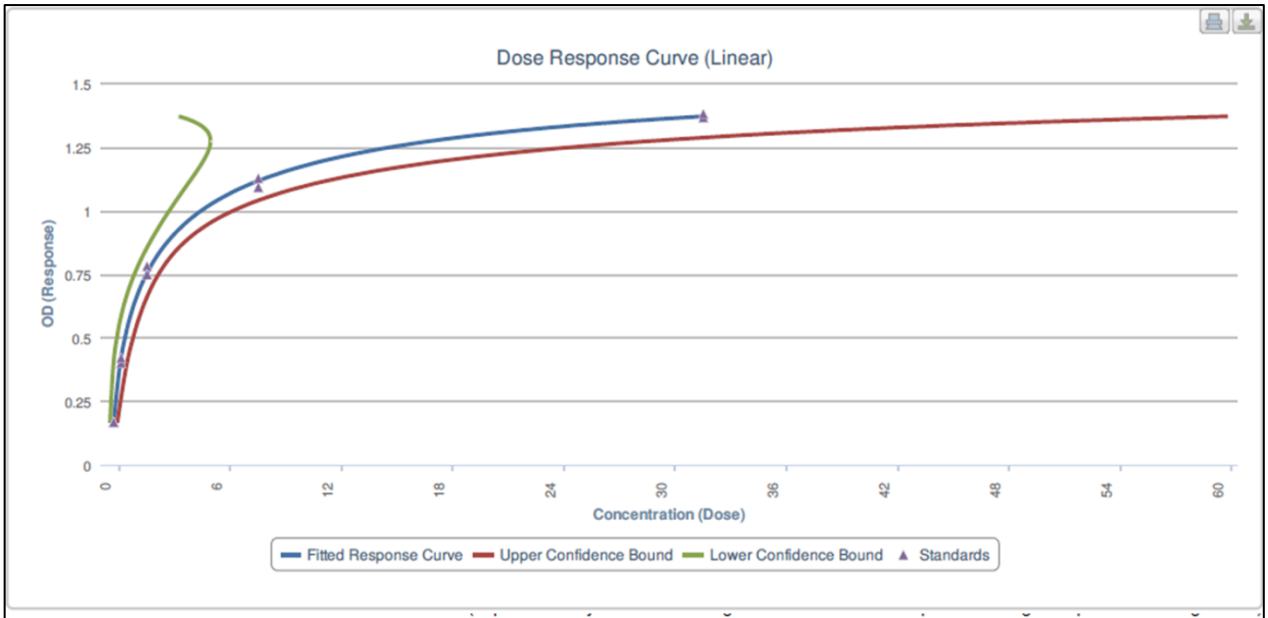
a	b	c	d
1.1912452	-0.5966726	1.440952	-0.2956337

R^2 value: 0.9962584

Well Category	Well	Well Position	Well Optical Density	Mean Optical Density	Mean Concentration	Confidence Interval
Unknown	U1	G1,G2	0.431,0.444	0.438	1.376	0.226 - 2.525
Unknown	U2	H1,H2	0.621,0.65	0.636	3.432	1.379 - 5.466
Unknown	U3	A3,A4	0.776,0.794	0.785	7.439	1.378 - 13.474
Unknown	U4	B3,B4	0.76,0.834	0.797	8.191	1.219 - 14.69
Unknown	U5	C3,C4	0.881,0.943	0.912	17.383	-4.292 - 37.827
Unknown	U6	D3,D4	0.984,0.992	0.988	31.665	-20.443 - 83.707
Unknown	U7	E3,E4	1.026,1.028	1.027	47.536	-43.041 - 138.104
Unknown	U8	F3,F4	0.882,0.911	0.897	15.097	-2.844 - 32.821
Unknown	U9	G3,G4	0.961,0.956	0.959	24.249	-11.636 - 60.119
Unknown	U10	H3,H4	0.978,1.007	0.993	33.511	-22.254 - 88.323
Standard	S1	B1,B2	0.01,0.091	0.051	0.2	N/A
Standard	S2	C1,C2	0.275,0.262	0.269	0.6	N/A
Standard	S3	D1,D2	0.498,0.517	0.508	2	N/A
Standard	S4	E1,E2	0.813,0.8	0.807	8	N/A
Standard	S5	F1,F2	0.985,0.988	0.987	32	N/A

	1	2	3	4
A	standard	standard	hc 12.5mg/ml	hc 12.5mg/ml
B	standard	standard	hc 25mg/ml	hc 25mg/ml
C	standard	standard	hc 50mg/ml	hc 50mg/ml
D	standard	standard	hc 100mg/ml	hc 100mg/ml
E	standard	standard	hc 200mg/ml	hc 200mg/ml
F	standard	standard	pfc 12.5mg/ml	pfc 12.5mg/ml
G	control	control	pfc 100mg/ml	pfc 100mg/ml
H	control	control	pfc 200mg/ml	pfc 200mg/ml

Figure 95- NE ELISA trial standard curve



Regression Formula: $y = d + \frac{a-d}{1+(x/c)^b}$

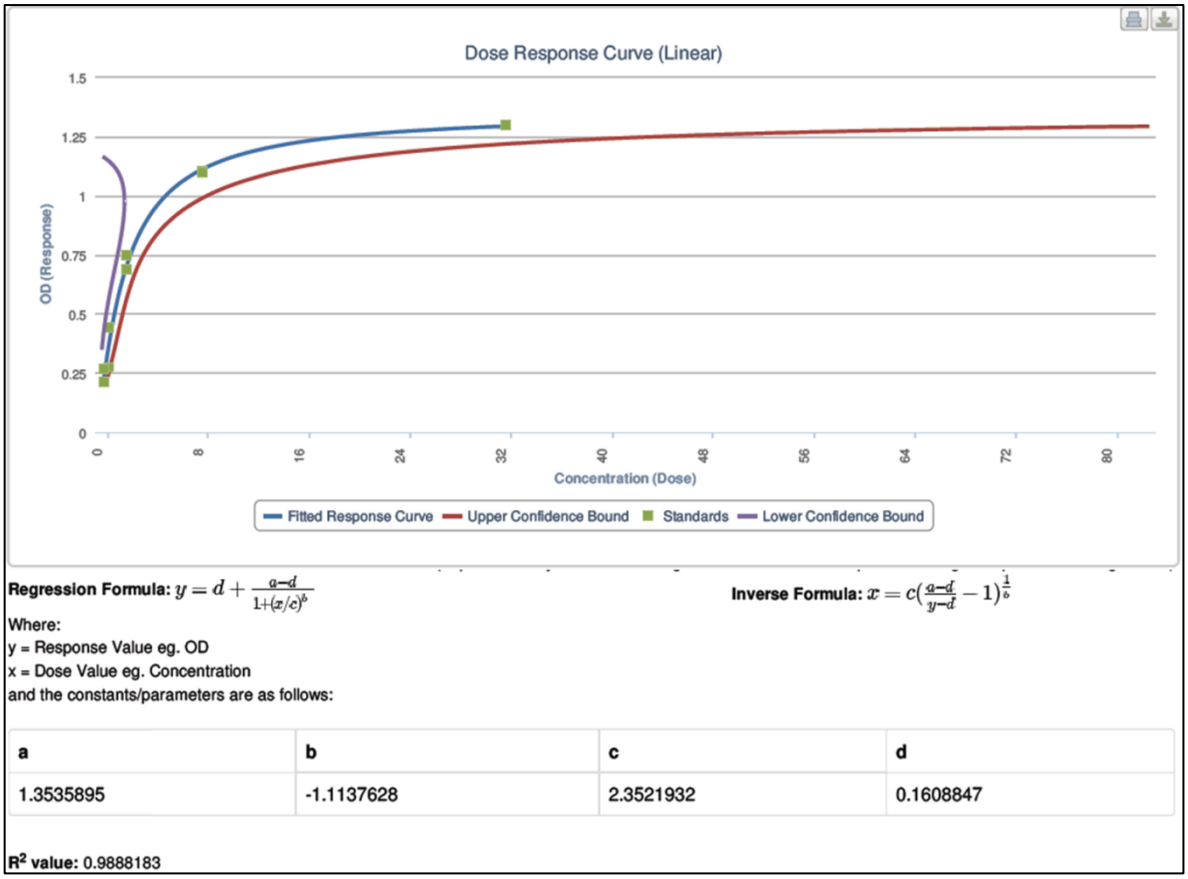
Inverse Formula: $x = c(\frac{a-d}{y-d} - 1)^{\frac{1}{b}}$

Where:
y = Response Value eg. OD
x = Dose Value eg. Concentration
and the constants/parameters are as follows:

a	b	c	d
1.6387645	-0.6023839	1.6095046	-0.2539012

R^2 value: 0.9990077

Figure 96- ELISA, HC NE standard curve



a	b	c	d
1.3535895	-1.1137628	2.3521932	0.1608847

Figure 97- ELISA, PFC NE standard curve

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LDN®

Instructions for use

Glutamate ELISA

REF BA E-2400

96 | +8 °C | IVD | CE

1. Intended use and principle of the test

Enzyme Immunoassay for the quantitative determination of L-Glutamate in urine, plasma, and serum samples.

After extraction and derivatisation Glutamate is quantitatively determined by ELISA.
The competitive ELISA uses the microtiter plate format. The antigen is bound to the solid phase of the microtiter plate. The acylated standards, controls and samples and the solid phase bound analyte compete for a fixed number of antiserum binding sites. When the system is in equilibrium, free antigen and free antigen-antiserum complexes are removed by washing. The antibody bound to the solid phase is detected by an anti-rabbit IgG-peroxidase conjugate using TMB as a substrate. The reaction is monitored at 450 nm.
Quantification of unknown samples is achieved by comparing their absorbance with a reference curve prepared with known standards.

2. Advice on handling the test

2.1 Reliability of the test results

In order to assure a reliable evaluation of the test results it must be conducted according to the instructions included and in accordance with current rules and guidelines (GLP, RiLiBÄK, etc.). Special attention must be paid to control checks for precision and correctness during the test: the results of these control checks have to be within the norm range. In case of significant discrepancies between the pre-set assay characteristics of this test and the actual results please contact the manufacturer of the test kit for further instructions.
It is recommended that each laboratory establishes its own reference intervals. The values reported in this test instruction are only indicative.
The results obtained with this test kit should not be taken as the sole reason for any therapeutic consequence but have to be correlated to other diagnostic tests and clinical observations.

2.2 Complaints

In case of complaints please submit to the manufacturer a written report containing all data as to how the test was conducted, the results received and a copy of the original test printout. Please contact the manufacturer to obtain a reclamation form and return it completely filled in to the manufacturer.

2.3 Warranty

This test kit was produced according to the latest developments in technology and subjected to stringent internal and external quality control checks. Any alteration of the test kit or the test procedure as well as the usage of reagents from different charges may have a negative influence on the test results and are therefore not covered by warranty. The manufacturer is not liable for damages incurred in transit.

2.4 Disposal

Residual substances and/or all remaining chemicals, reagents and ready for use solutions, are special refuse. The disposal is subject to the laws and regulations of the federation and the countries. About the removal of special refuse the responsible authorities or refuse disposal enterprises inform. The disposal of the kit must be made according to the national official regulations. Legal basis for the disposal of special refuse is the cycle economic- and waste law.
The appropriate safety data sheets of the individual products are available on the homepage. The safety data sheets correspond to the standard: ISO 11014-1.

2.5 Interference

Do not mix reagents and solutions from different lots. Consider different transport and storage conditions. Inappropriate handling of test samples or deviations from the test regulation can the results affect. Use no kit components beyond the expiration date. Avoid microbiological contamination of the reagents and the washing water. Consider incubation periods and wash references.

2.6 Precautions

Observe the incubation periods and washing instructions. Never pipette by mouth and avoid contact of reagents and specimens with skin. No smoking, eating or drinking in areas where samples or kit test tubes are handled. When working with kit components or samples, always wear protective gloves and wash your hand thoroughly as soon as you have finished the work. Avoid spraying of any kind. Avoid any skin contact with reagents. Use protective clothing and disposable gloves. All steps have to be performed according to the protocol. Optimal test results are only obtained when using calibrated pipettes. Sodium azide could react with lead and copper tubes and may form highly explosive metal azide. When clearing up, rinse thoroughly with large volumes of water to prevent such formation.
All reagents of this testkit which contain human or animal serum or plasma have been tested and confirmed negative for HIV I/II, HbsAg and HCV by FDA approved procedures.
All reagents, however, should be treated as potential biohazards in use and for disposal.

3. Storage and stability

Store the reagents at 2 - 8 °C until expiration date. Do not use components beyond the expiry date indicated on the kit labels. Do not mix various lots of any kit component within an individual assay.

4.1 Contents of the kit

BA D-0024	REAC-PLATE	**Reaction Plate**	1 x 96 wells	ready for use
BA D-0090	FOILS	**Adhesive Foil**	1 x 4	ready for use
BA E-0030	WASH-CONC 50x	**Wash Buffer Concentrate**	1 x 20 mL	concentrate, dilute content with dist. water to a final volume of 1000 mL
BA E-0040	CONJUGATE	**Enzyme Conjugate**	1 x 12 mL	ready for use, anti-rabbit IgG conjugated with peroxidase
BA E-0055	SUBSTRATE	**Substrate**	1 x 12 mL	ready for use, containing a solution of tetramethylbenzidine (TMB)
BA E-0080	STOP-SOLN	**Stop Solution**	1 x 12 mL	ready for use, containing 0.25 M H_2SO_4
BA E-2401	STANDARD A	**Standard A**	1 x 4 mL	ready for use
BA E-2402	STANDARD B	**Standard B**	1 x 4 mL	ready for use
BA E-2403	STANDARD C	**Standard C**	1 x 4 mL	ready for use
BA E-2404	STANDARD D	**Standard D**	1 x 4 mL	ready for use
BA E-2405	STANDARD E	**Standard E**	1 x 4 mL	ready for use
BA E-2406	STANDARD F	**Standard F**	1 x 4 mL	ready for use
BA E-2410	AS-GLUT	**Glutamate Antiserum**	1 x 6 mL	from rabbit, ready for use, blue coloured, blue screw cap
BA E-2413	ASSAY-BUFF	**Assay Buffer**	1 x 20 mL	ready for use
BA E-2428	EQUA-REAG	**Equalizing Reagent**	1 x	lyophilized
BA E-2431	96 GLUT	**Glutamate Microtiter Strips**	1 x 96 wells	12 strips, 8 wells each, break apart, precoated
BA E-2442	EXTRACT-PLATE 48	**Extraction Plate**	2 x 48 wells	ready for use
BA E-2446	D-REAGENT	**D-Reagent**	1 x 4 mL	ready for use
BA E-2451	CONTROL 1	**Control 1**	1 x 4 mL	ready for use
BA E-2452	CONTROL 2	**Control 2**	1 x 4 mL	ready for use
BA E-2458	Q-BUFFER	**Q-Buffer**	1 x 20 mL	ready for use
BA E-2460	DILUENT	**Diluent**	1 x 20 mL	ready for use
BA E-2787	NAOH	**NaOH**	1 x 2 mL	ready for use

4.2 Additional materials and equipment required but not provided with the kit

- Calibrated variable precision micropipettes (e.g. 10-100 µL / 100-1000 µL)
- Polystyrene tubes and suitable rack
- Microtiter plate washing device
- ELISA reader capable of reading absorbance at 450 nm
- Shaker (shaking amplitude 3mm; approx. 600 rpm)
- Absorbent material (paper towel)
- Distilled water
- Vortex mixer

5. Sample collection and storage

Urine
Spontaneous or 24-hour urine, collected in a bottle containing 10-15 mL of 6 M HCl, should be used.
Storage: for a longer period (up to 6 months) at -20°C.
Repeated freezing and thawing should be avoided.

Serum or Plasma
Serum or EDTA-Plasma can be used. Fasting specimens or pre-feed specimens for children (2-3 hours after last meal) are advised. Do not use haemolytic or lipemic samples.
Storage: up to 24 hours at 2 - 8°C, for longer period (up to 6 month) at - 20°C.
Repeated freezing and thawing should be avoided.

6. Test procedure

Allow all reagents and samples to reach room temperature. Duplicate determinations are recommended.

6.1 Preparation of reagents

Wash Buffer
Dilute the 20 mL Wash Buffer Concentrate with distilled water to a final volume of 1000 mL.
Storage: up to 6 months 2–8°C.

Equalizing Reagent
Reconstitute the Equalizing Reagent with **12.5 mL** of **Assay Buffer.**
Reconstituted Equalizing Reagent which is not used immediately has to be stored in aliquotes at -20°C and may be thawed only once.

6.2 Preparation of samples

The Glutamate ELISA is a flexible test system for various biological sample types and volumes. It is not possible to give a general advice how to prepare the samples. However, the following basics should help the researcher to adapt the protocol to his specific needs:

- **Urine samples** with creatinine >200 mg/dL should be diluted 1:3 (e.g. 100 µL urine + 200 µL distilled water) before starting the Extraction step. **The results of the diluted urine samples have to be corrected for the dilution factor.**
- **Serum/plasma samples** should always be pre-diluted 1:5 (e.g. 100 µL serum/plasma + 400 µL distilled water). Serum values of Glutamate are higher than for urine. The pre-dilution step makes sure that the sample is measured in the linear range of the standard curve. **The results have to be corrected for the dilution factor.**
- Avoid excess of acid: excess of acid might exceed the buffer capacity of the dilution buffer. A **pH of 5.0** during the extraction is mandatory.
- It is advisable to perform a **Proof of Principle** to determine the recovery of glutamate in the samples. Prepare a stock solution of glutamate. Add small amounts (to change the native sample matrix as less as possible) of the stock solutions to the sample matrix and check the recovery.
- The sample volume determines the sensitivity of this test. Determine the sample volume needed to determine glutamate in your sample by testing different amounts of sample volumes.

If you need any support in establishing a protocol for your specific purposes, do not hesitate to contact the manufacturer directly!

6.3 Extraction

1.	Pipette **100 µL** of the **standards, controls** and **samples (serum 1:5 diluted)** into the appropriate wells of the **Extraction Plate.**
2.	Add **100 µL** of the **Diluent** to all wells. Cover plate with **Adhesive Foil** and **shake** for **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
3.	Use **25 µl** for the subsequent derivatization!

6.4 Derivatization

1.	Pipette **25 µL** of the **extracted standards, controls** and **samples** into the appropriate wells of the **Reaction Plate.**
2.	Pipette **10 µL** of **NaOH** into all wells.
3.	Pipette **50 µL** of the **Equalizing Reagent** into all wells.
4.	Pipette **10 µL** of the **D-Reagent** into all wells.
5.	Cover plate with **Adhesive Foil** and shake for **2 hours** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
6.	Pipette **75 µL** of the **Q-Buffer** into all wells.
7.	Shake for **10 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
8.	**Use 25 µl for the ELISA!**

Figure 98- Glutamate ELISA instruction manual 1/2

6.5 Glutamate ELISA

1.	Pipette **25 µL** of the **prepared standards, controls and samples** into the appropriate wells of the **Glutamate Microtiter Strips.**
2.	Pipette **50 µL** of the **Glutamate Antiserum** into all wells and mix shortly.
3.	Cover plate with **Adhesive Foil** and incubate for **15 - 20 hours** (overnight) at **2 – 8 °C.** ***Alternatively*** *incubate* ***2 hours*** *at* ***RT*** *(20-25°C) on a* ***shaker*** *(approx. 600rpm).*
4.	Remove the foil and discard. Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer.** Blot dry by tapping the inverted plate on absorbent material.
5.	Pipette **100 µL** of the **Enzyme Conjugate** into all wells.
6.	Incubate for **30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm).
7.	Discard or aspirate the contents of the wells and **wash** each well **3 times** thoroughly with **300 µL Wash Buffer.** Blot dry by tapping the inverted plate on absorbent material.
8. ⚠	Pipette **100 µL** of the **Substrate** into all wells and incubate for **20-30 min** at **RT** (20-25°C) on a shaker (approx. 600 rpm). ***Avoid exposure to direct sun light!***
9.	Add **100 µL** of the **Stop Solution** to each well and shake the microtiter plate to ensure a homogeneous distribution of the solution.
10.	**Read** the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to **450 nm** and a reference wavelength between 620 nm and 650 nm.

7. Calculation of results

	Concentration of the standards					
Standard	**A**	**B**	**C**	**D**	**E**	**F**
Glutamate (µg/mL)	0	0.6	2	6	20	60
Glutamate (µmol/L)	0	4.08	13.6	40.8	136	408
Conversion:	Glutamate (µg/mL) x 6.8 = Glutamate (µmol/L)					

The calibration curve is obtained by plotting the absorbance readings (calculate the mean absorbance) of the standards (linear, y-axis) against the corresponding standard concentrations (logarithmic, x-axis).
Use non-linear regression for curve fitting (e.g. spline, 4- parameter, akima).

Serum/plasma
The read concentrations of **plasma samples** have to be **multiplied by 5.**

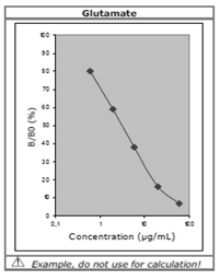
Urine samples and controls:
The concentrations of the samples and controls can be read directly from the standard curve.
Diluted urine samples (refer to 6.2) have to be **multiplied by 3.**

7.1 Quality control
It is recommended to use control samples according to state and federal regulations. Use controls at both normal and pathological levels. The kit or other commercial controls should fall within established confidence limits. The confidence limits of the kit controls are indicated on the QC Report.

7.2 Calibration
The binding of the antisera and of the enzyme conjugate and the activity of the enzyme are temperature dependent, and the extinction values may vary if a thermostat is not used. The higher the temperature, the higher the extinction values will be. Corresponding variations also apply to the incubation times. The optimal temperature during the Enzyme Immunoassay is between 20-25°C.

⚠ *In case of overflow, read the absorbance of the solution in the wells within 10 minutes, using a microplate reader set to 405 nm*

7.3 Typical calibration curve



⚠ *Example, do not use for calculation!*

8. Assay characteristics

Expected Reference Values		**Glutamate**
	Urine (spontaneous)	5 – 100 µmol/g creatinine
	Serum / Plasma	15 – 220 µmol/L (2.2 – 30 µg/mL)

Analytical Sensitivity (Limit of Detection)	**Glutamate**
	0.3 µg/mL

	Substance	**Cross Reactivity (%)**
Analytical Specificity (Cross Reactivity)	Glutamate	100
	Glutamine	< 0.01
	Aspartate	0.09
	Glycine	< 0.01
	Alanine	< 0.01
	5-aminovaleric acid	< 0.01

Precision

Intra-Assay			**Inter-Assay**		
Sample	Range (µg/mL)	CV (%)	Sample	Range (µg/mL)	CV (%)
1 (n = 25)	6.9 ± 0.5	7.3	1 (n = 19)	0.8 ± 0.18	19
2 (n = 25)	16.0 ± 1.0	6.3	2 (n = 19)	9 ± 1.2	13

Linearity		Range	Serial dilution up to	Range (%)
	Glutamate (urine)	0.61 – 47 µg/mL	1:64	83 - 111
	Glutamate (serum)	1 – 24 µg/mL	1:20	82 - 98

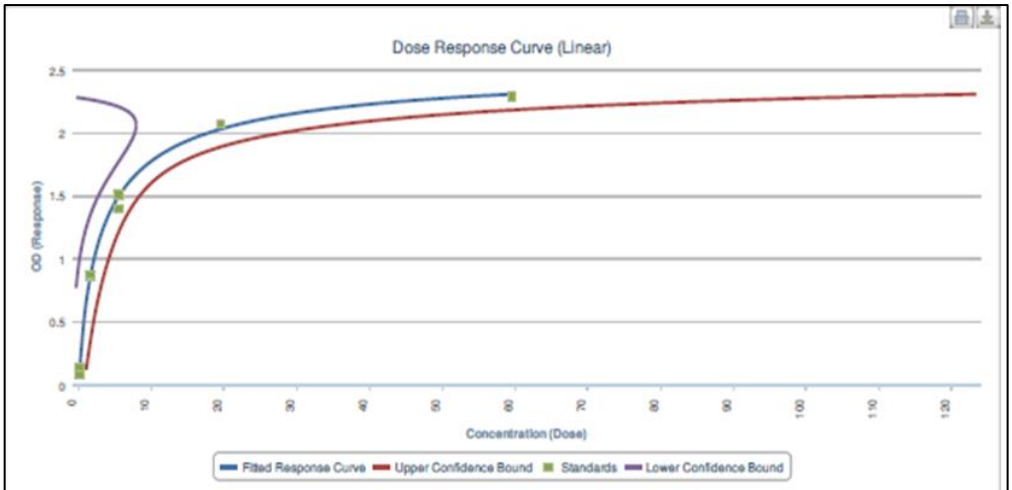
Recovery		Mean (%)	Range (%)	% Recovery after spiking
	Glutamate (urine)	98	83 - 105	
	Glutamate (serum)	99	96 - 104	

⚠ ***For current literature, information about clinical significance or any other information please contact your local supplier.***

Symbols:

	Storage temperature		Manufacturer		Contains sufficient for <n> tests
	Expiry date	LOT	Batch code	IVD	For in-vitro diagnostic use only!
	Consult instructions for use	CONT	Content	CE	CE labelled
	Caution	REF	Catalogue number	RUO	For research use only!

Figure 99- Glutamate ELISA instruction manual 2/2



Regression Formula: $y = d + \frac{a-d}{1+(x/c)^b}$

Inverse Formula: $x = c\left(\frac{a-d}{y-d} - 1\right)^{\frac{1}{b}}$

Where:
y = Response Value eg. OD
x = Dose Value eg. Concentration
and the constants/parameters are as follows:

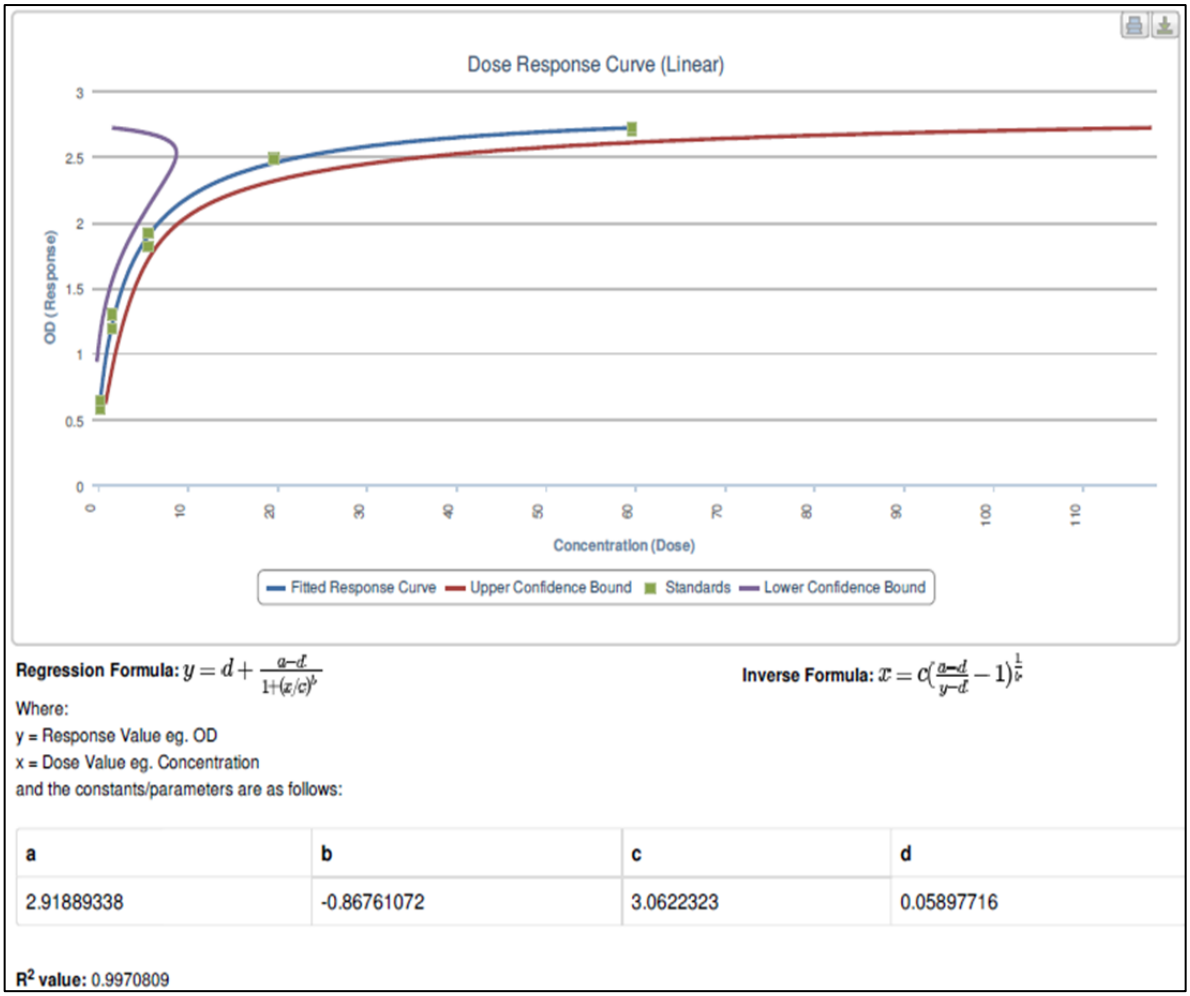
a	b	c	d
2.5690372	-0.7342802	2.0159478	-0.8886692

R^2 value: 0.9970958

Well Category	Well	Well Position	Well Optical Density	Mean Optical Density	Mean Concentration	Confidence Interval
Unknown	U1	G1,G2	0.511,0.632	0.572	1.322	-0.115 - 2.746
Unknown	U2	H1,H2	1.258,1.306	1.282	4.112	1.691 - 6.525
Unknown	U3	A3,A4	0.563,0.563	0.563	1.298	-0.122 - 2.718
Unknown	U4	B3,B4	1.495,1.495	1.495	5.97	3.147 - 8.794
Unknown	U5	C3,C4	1.969,1.969	1.969	16.889	7.988 - 25.79
Unknown	U6	D3,D4	2.276,2.276	2.276	51.505	0.475 - 102.536
Unknown	U7	E3,E4	2.348,2.348	2.348	77.97	-14.743 - 170.684
Unknown	U8	F3,F4	2.145,2.145	2.145	29.396	7.713 - 51.079
Unknown	U9	G3,G4	2.355,2.355	2.355	81.704	-17.306 - 180.713
Unknown	U10	H3,H4	2.204,2.204	2.204	37.008	5.973 - 68.044
Standard	S1	B1,B2	0.14,0.083	0.112	0.6	N/A
Standard	S2	C1,C2	0.868,0.854	0.861	2	N/A
Standard	S3	D1,D2	1.51,1.395	1.453	6	N/A
Standard	S4	E1,E2	2.073,2.072	2.073	20	N/A
Standard	S5	F1,F2	2.283,2.289	2.286	60	N/A

	1	2	3	4
A	standard	standard	hc 1mg/ml	hc 1mg/ml
B	standard	standard	hc 5mg/ml	hc 5mg/ml
C	standard	standard	hc 10mg/ml	hc 10mg/ml
D	standard	standard	hc 50mg/ml	hc 50mg/ml
E	standard	standard	hc 200mg/ml	hc 200mg/ml
F	standard	standard	pfc 20mg/ml	pfc 20mg/ml
G	control	control	pfc 200mg/ml	pfc 200mg/ml
H	control	control	pfc 20mg/ml glu recov	pfc 20mg/ml glu recov

Figure 100- Glutamate ELISA trial standard curve



Regression Formula: $y = d + \frac{a-d}{1+(x/c)^b}$

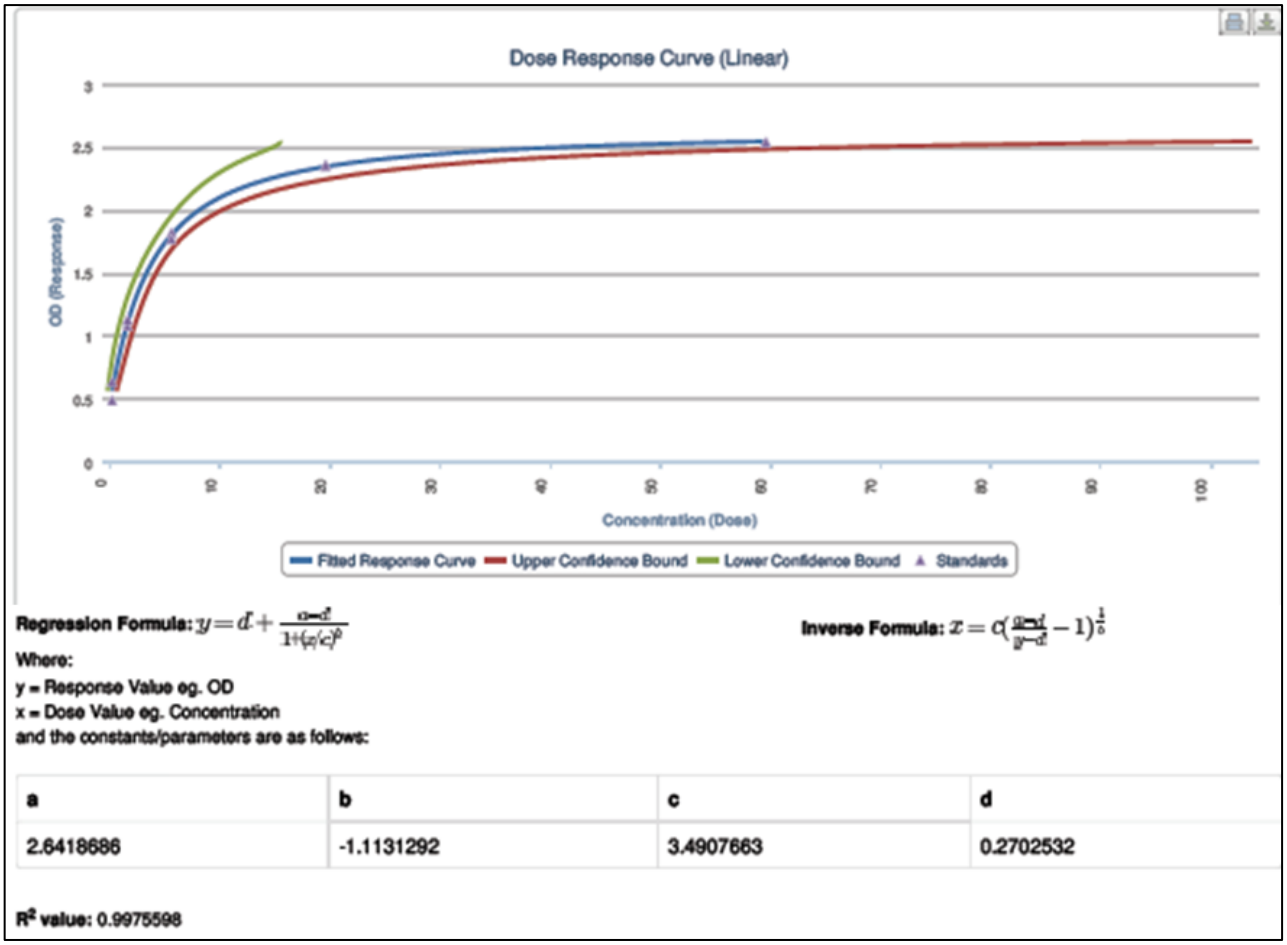
Inverse Formula: $x = c\left(\frac{a-d}{y-d} - 1\right)^{\frac{1}{b}}$

Where:
y = Response Value eg. OD
x = Dose Value eg. Concentration
and the constants/parameters are as follows:

a	b	c	d
2.91889338	-0.86761072	3.0622323	0.05897716

R^2 value: 0.9970809

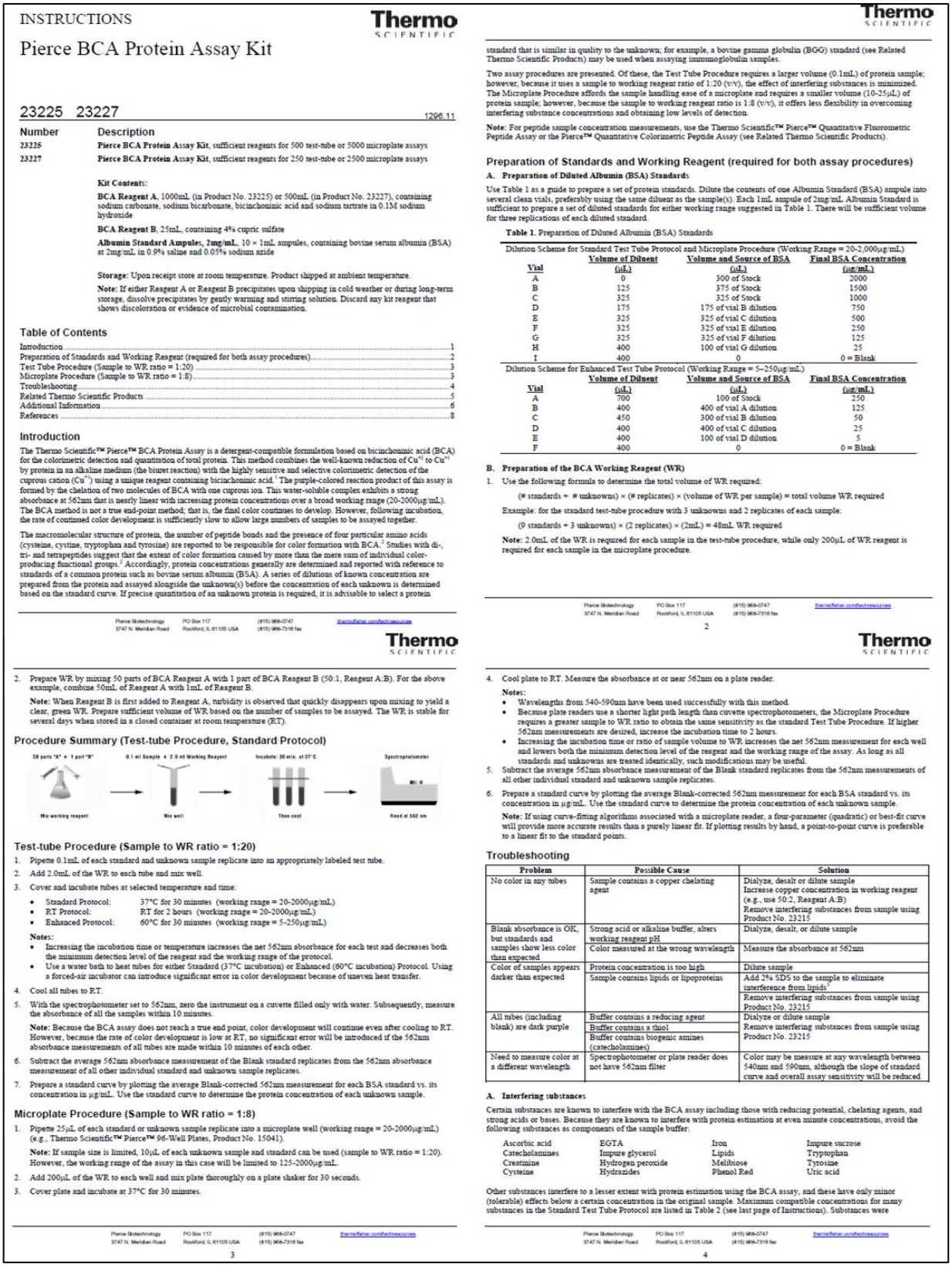
Figure 101- ELISA, HC Glu standard curve



a	b	c	d
2.6418686	-1.1131292	3.4907663	0.2702532

R^2 value: 0.9975598

Figure 102- ELISA, PFC Glu standard curve



INSTRUCTIONS

Thermo SCIENTIFIC

Pierce BCA Protein Assay Kit

23225 23227

1296.11

Number	Description
23225	**Pierce BCA Protein Assay Kit**, sufficient reagents for 500 test-tube or 5000 microplate assays
23227	**Pierce BCA Protein Assay Kit**, sufficient reagents for 250 test-tube or 2500 microplate assays

Kit Contents:

BCA Reagent A, 1000mL (in Product No. 23225) or 500mL (in Product No. 23227), containing sodium carbonate, sodium bicarbonate, bicinchoninic acid and sodium tartrate in 0.1M sodium hydroxide

BCA Reagent B, 25mL, containing 4% cupric sulfate

Albumin Standard Ampules, 2mg/mL, 10 × 1mL ampules, containing bovine serum albumin (BSA) at 2mg/mL in 0.9% saline and 0.05% sodium azide

Storage: Upon receipt store at room temperature. Product shipped at ambient temperature.

Note: If either Reagent A or Reagent B precipitates upon shipping in cold weather or during long-term storage, dissolve precipitates by gently warming and stirring solution. Discard any kit reagent that shows discoloration or evidence of microbial contamination.

Table of Contents

Introduction

The Thermo Scientific™ Pierce™ BCA Protein Assay is a detergent-compatible formulation based on bicinchoninic acid (BCA) for the colorimetric detection and quantitation of total protein. This method combines the well-known reduction of Cu^{+2} to Cu^{+1} by protein in an alkaline medium (the biuret reaction) with the highly sensitive and selective colorimetric detection of the cuprous cation (Cu^{+1}) using a unique reagent containing bicinchoninic acid.[1] The purple-colored reaction product of this assay is formed by the chelation of two molecules of BCA with one cuprous ion. This water-soluble complex exhibits a strong absorbance at 562nm that is nearly linear with increasing protein concentrations over a broad working range (20-2000µg/mL). The BCA method is not a true end-point method; that is, the final color continues to develop. However, following incubation, the rate of continued color development is sufficiently slow to allow large numbers of samples to be assayed together.

The macromolecular structure of protein, the number of peptide bonds and the presence of four particular amino acids (cysteine, cystine, tryptophan and tyrosine) are reported to be responsible for color formation with BCA.[2] Studies with di-, tri- and tetrapeptides suggest that the extent of color formation caused by more than the mere sum of individual color-producing functional groups.[3] Accordingly, protein concentrations generally are determined and reported with reference to standards of a common protein such as bovine serum albumin (BSA). A series of dilutions of known concentration are prepared from the protein and assayed alongside the unknown(s) before the concentration of each unknown is determined based on the standard curve. If precise quantitation of an unknown protein is required, it is advisable to select a protein

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standard that is similar in quality to the unknown; for example, a bovine gamma globulin (BGG) standard (see Related Thermo Scientific Products) may be used when assaying immunoglobulin samples.

Two assay procedures are presented. Of these, the Test Tube Procedure requires a larger volume (0.1mL) of protein sample; however, because it uses a sample to working reagent ratio of 1:20 (v/v), the effect of interfering substances is minimized. The Microplate Procedure affords the sample handling ease of a microplate and requires a smaller volume (10-25µL) of protein sample; however, because the sample to working reagent ratio is 1:8 (v/v), it offers less flexibility in overcoming interfering substance concentrations and obtaining low levels of detection.

Note: For peptide sample concentration measurements, use the Thermo Scientific™ Pierce™ Quantitative Fluorometric Peptide Assay or the Pierce™ Quantitative Colorimetric Peptide Assay (see Related Thermo Scientific Products).

Preparation of Standards and Working Reagent (required for both assay procedures)

A. Preparation of Diluted Albumin (BSA) Standards

Use Table 1 as a guide to prepare a set of protein standards. Dilute the contents of one Albumin Standard (BSA) ampule into several clean vials, preferably using the same diluent as the sample(s). Each 1mL ampule of 2mg/mL Albumin Standard is sufficient to prepare a set of diluted standards for either working range suggested in Table 1. There will be sufficient volume for three replications of each diluted standard.

Table 1. Preparation of Diluted Albumin (BSA) Standards

Dilution Scheme for Standard Test Tube Protocol and Microplate Procedure (Working Range = 20-2,000µg/mL)

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (µg/mL)
A	0	300 of Stock	2000
B	125	375 of Stock	1500
C	325	325 of Stock	1000
D	175	175 of vial B dilution	750
E	325	325 of vial C dilution	500
F	325	325 of vial E dilution	250
G	325	325 of vial F dilution	125
H	400	100 of vial G dilution	25
I	400	0	0 = Blank

Dilution Scheme for Enhanced Test Tube Protocol (Working Range = 5–250µg/mL)

Vial	Volume of Diluent (µL)	Volume and Source of BSA (µL)	Final BSA Concentration (µg/mL)
A	700	100 of Stock	250
B	400	400 of vial A dilution	125
C	450	300 of vial B dilution	50
D	400	400 of vial C dilution	25
E	400	100 of vial D dilution	5
F	400	0	0 = Blank

B. Preparation of the BCA Working Reagent (WR)

1. Use the following formula to determine the total volume of WR required:

   (# standards + # unknowns) × (# replicates) × (volume of WR per sample) = total volume WR required

   Example: for the standard test-tube procedure with 3 unknowns and 2 replicates of each sample:

   (9 standards + 3 unknowns) × (2 replicates) × (2mL) = 48mL WR required

   Note: 2.0mL of the WR is required for each sample in the test-tube procedure, while only 200µL of WR reagent is required for each sample in the microplate procedure.

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2. Prepare WR by mixing 50 parts of BCA Reagent A with 1 part of BCA Reagent B (50:1, Reagent A:B). For the above example, combine 50mL of Reagent A with 1mL of Reagent B.

   Note: When Reagent B is first added to Reagent A, turbidity is observed that quickly disappears upon mixing to yield a clear, green WR. Prepare sufficient volume of WR based on the number of samples to be assayed. The WR is stable for several days when stored in a closed container at room temperature (RT).

Procedure Summary (Test-tube Procedure, Standard Protocol)

Test-tube Procedure (Sample to WR ratio = 1:20)

1. Pipette 0.1mL of each standard and unknown sample replicate into an appropriately labeled test tube.
2. Add 2.0mL of the WR to each tube and mix well.
3. Cover and incubate tubes at selected temperature and time:
   - Standard Protocol: 37°C for 30 minutes (working range = 20-2000µg/mL)
   - RT Protocol: RT for 2 hours (working range = 20-2000µg/mL)
   - Enhanced Protocol: 60°C for 30 minutes (working range = 5-250µg/mL)

   Notes:
   - Increasing the incubation time or temperature increases the net 562nm absorbance for each test and decreases both the minimum detection level of the reagent and the working range of the protocol.
   - Use a water bath to heat tubes for either Standard (37°C incubation) or Enhanced (60°C incubation) Protocol. Using a forced-air incubator can introduce significant error in color development because of uneven heat transfer.
4. Cool all tubes to RT.
5. With the spectrophotometer set to 562nm, zero the instrument on a cuvette filled only with water. Subsequently, measure the absorbance of all the samples within 10 minutes.

   Note: Because the BCA assay does not reach a true end point, color development will continue even after cooling to RT. However, because the rate of color development is low at RT, no significant error will be introduced if the 562nm absorbance measurements of all tubes are made within 10 minutes of each other.
6. Subtract the average 562nm absorbance measurement of the Blank standard replicates from the 562nm absorbance measurement of all other individual standard and unknown sample replicates.
7. Prepare a standard curve by plotting the average Blank-corrected 562nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

Microplate Procedure (Sample to WR ratio = 1:8)

1. Pipette 25µL of each standard or unknown sample replicate into a microplate well (working range = 20-2000µg/mL) (e.g., Thermo Scientific™ Pierce™ 96-Well Plates, Product No. 15041).

   Note: If sample size is limited, 10µL of each unknown sample and standard can be used (sample to WR ratio = 1:20). However, the working range of the assay in this case will be limited to 125-2000µg/mL.
2. Add 200µL of the WR to each well and mix plate thoroughly on a plate shaker for 30 seconds.
3. Cover plate and incubate at 37°C for 30 minutes.

4. Cool plate to RT. Measure the absorbance at or near 562nm on a plate reader.

   Notes:
   - Wavelengths from 540-590nm have been used successfully with this method.
   - Because plate readers use a shorter light path length than cuvette spectrophotometers, the Microplate Procedure requires a greater sample to WR ratio to obtain the same sensitivity as the standard Test Tube Procedure. If higher 562nm measurements are desired, increase the incubation time to 2 hours.
   - Increasing the incubation time or ratio of sample volume to WR increases the net 562nm measurement for each well and lowers both the minimum detection level of the reagent and the working range of the assay. As long as all standards and unknowns are treated identically, such modifications may be useful.
5. Subtract the average 562nm absorbance measurement of the Blank standard replicates from the 562nm measurements of all other individual standard and unknown sample replicates.
6. Prepare a standard curve by plotting the average Blank-corrected 562nm measurement for each BSA standard vs. its concentration in µg/mL. Use the standard curve to determine the protein concentration of each unknown sample.

   Note: If using curve-fitting algorithms associated with a microplate reader, a four-parameter (quadratic) or best-fit curve will provide more accurate results than a purely linear fit. If plotting results by hand, a point-to-point curve is preferable to a linear fit to the standard points.

Troubleshooting

Problem	Possible Cause	Solution
No color in any tubes	Sample contains a copper chelating agent	Dialyze, desalt or dilute sample Increase copper concentration in working reagent (e.g., use 50:2, Reagent A:B) Remove interfering substances from sample using Product No. 23215
Blank absorbance is OK, but standards and samples show less color than expected	Strong acid or alkaline buffer, alters working reagent pH	Dialyze, desalt, or dilute sample
	Color measured at the wrong wavelength	Measure the absorbance at 562nm
Color of samples appears darker than expected	Protein concentration is too high	Dilute sample
	Sample contains lipids or lipoproteins	Add 2% SDS to the sample to eliminate interference from lipids[4] Remove interfering substances from sample using Product No. 23215
All tubes (including blank) are dark purple	Buffer contains a reducing agent	Dialyze or dilute sample Remove interfering substances from sample using Product No. 23215
	Buffer contains a thiol	
	Buffer contains biogenic amines (catecholamines)	
Need to measure color at a different wavelength	Spectrophotometer or plate reader does not have 562nm filter	Color may be measure at any wavelength between 540nm and 590nm, although the slope of standard curve and overall assay sensitivity will be reduced.

A. Interfering Substances

Certain substances are known to interfere with the BCA assay including those with reducing potential, chelating agents, and strong acids or bases. Because they are known to interfere with protein estimation at even minute concentrations, avoid the following substances as components of the sample buffer:

Ascorbic acid	EGTA	Iron	Impure sucrose
Catecholamines	Impure glycerol	Lipids	Tryptophan
Creatinine	Hydrogen peroxide	Melibiose	Tyrosine
Cysteine	Hydrazides	Phenol Red	Uric acid

Other substances interfere to a lesser extent with protein estimation using the BCA assay, and these have only minor (tolerable) effects below a certain concentration in the original sample. Maximum compatible concentrations for many substances in the Standard Test Tube Protocol are listed in Table 2 (see last page of Instructions). Substances were

Figure 103- BCA protein assay instruction manual 1/2

compatible at the indicated concentration in the Standard Test Tube Protocol if the error in protein concentration estimation caused by the presence of the substance was less than or equal to 10%. The substances were tested using WR prepared immediately before each experiment. Blank-corrected 562nm absorbance measurements (for a 1000µg/mL BSA standard + substance) were compared to the net 562nm measurements of the same standard prepared in 0.9% saline. Maximum compatible concentrations will be lower In the Microplate Procedure where the sample to WR ratio is 1:8 (v/v).

Furthermore, it is possible to have a substance additive affect such that even though a single component is present at a concentration below its listed compatibility, a sample buffer containing a combination of substances could interfere with the assay.

B. Strategies for eliminating or minimizing the effects of interfering substances

The effects of interfering substances in the Pierce BCA Protein Assay may be eliminated or overcome by one of several methods.

- Remove the interfering substance by dialysis or gel filtration.
- Dilute the sample until the substance no longer interferes. This strategy is effective only if the starting protein concentration is sufficient to remain in the working range of the assay upon dilution.
- Precipitate the proteins in the sample with acetone or trichloroacetic acid (TCA). The liquid containing the substance that interfered is discarded and the protein pellet is easily solubilized in ultrapure water or directly in the alkaline BCA WR.[4] A protocol detailing this procedure is available from our website. Alternatively, Product No. 23215 may be used (see Related Thermo Scientific Products).
- Increase the amount of copper in the WR (prepare WR as 50:2 or 50:3, Reagent A:B), which may eliminate interference by copper-chelating agents.

Note: For greatest accuracy, the protein standards must be treated identically to the sample(s).
Note: The Thermo Scientific™ Pierce™ Detergent Compatible Bradford Assay Kit (Product No. 23246) is an alternative related product compatible with a wide range of detergents.

Related Thermo Scientific Products

15041	**Pierce 96-Well Plates**, 100/pkg.
15075	**Reagent Reservoirs**, 200/pkg.
15036	**Sealing Tape for 96-Well Plates**, 100/pkg.
23209	**Albumin Standard Ampules, 2mg/mL**, 10 × 1mL ampules, containing bovine serum albumin (BSA)
23208	**Pre-Diluted Protein Assay Standards: Bovine Serum Albumin (BSA) Set**, 7 × 3.5mL
23212	**Bovine Gamma Globulin Standard, 2mg/mL**, 10 × 1mL ampules
23213	**Pre-Diluted Protein Assay Standards**, (BGG) Set, 7 × 3.5mL aliquots
23246	**Pierce Detergent Compatible Bradford Assay Kit**
23235	**Pierce Micro BCA Protein Assay Kit**
23290	**Pierce Quantitative Fluorometric Peptide Assay**
23275	**Pierce Quantitative Colorimetric Peptide Assay**
23236	**Coomassie Plus™ (Bradford) Assay Kit**
23215	**Compat-Able™ Protein Assay Preparation Reagent Set**
23250	**Pierce BCA Protein Assay Kit–Reducing Agent Compatible**

Additional Information

A. Please visit our website for additional information including the following items:

- Tech Tip #8: Eliminate interfering substances from samples for BCA Protein Assay

B. Alternative Total Protein Assay Reagents

If interference by a reducing substance or metal-chelating substance contained in the sample cannot be overcome, try the Coomassie Plus (Bradford) Assay Kit (Product No. 23236), which is less sensitive to such substances.

C. Cleaning and Re-using Glassware

Exercise care when re-using glassware. All glassware must be cleaned and given a thorough final rinse with ultrapure water.

D. Response characteristics for different proteins

Each of the commonly used total protein assay methods exhibits some degree of varying response toward different proteins. These differences relate to amino acid sequence, pI, structure and the presence of certain side chains or prosthetic groups that can dramatically alter the protein's color response. Most protein assay methods use BSA or immunoglobulin (IgG) as the standard against which the concentration of protein in the sample is determined (Figure 1). However, if great accuracy is required, prepare the standard curve from a pure sample of the target protein.

Typical protein-to-protein variation in color response is listed in Table 3. All proteins were tested at 1000µg/mL using the 30-minute/37°C Test Tube Protocol. The average net color response for BSA was normalized to 1.00 and the average net color response of the other proteins is expressed as a ratio to the response of BSA.

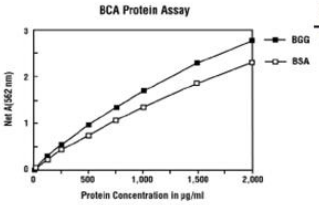

Figure 1. Typical color response curves for BSA and BGG using the Standard Test Tube Protocol (37°C/30-minute incubation).

Table 3. Protein-to-protein variation. Absorbance ratios (562nm) for proteins relative to BSA using the Standard Test Tube Protocol.

Ratio = (Avg "test" net Abs.) / (avg. BSA net Abs.)

Protein Tested	Ratio
Albumin, bovine serum	1.00
Aldolase, rabbit muscle	0.85
α-Chymotrypsinogen, bovine	1.14
Cytochrome C, horse heart	0.83
Gamma globulin, bovine	1.11
IgG, bovine	1.21
IgG, human	1.09
IgG, mouse	1.15
IgG, rabbit	1.12
IgG, sheep	1.17
Insulin, bovine pancreas	1.08
Myoglobin, horse heart	0.74
Ovalbumin	0.93
Transferrin, human	0.89
	1.02
Standard Deviation	0.15
Coefficient of Variation	14.7%

Table 2. Compatible substance concentrations in the Thermo Scientific Pierce BCA Protein Assay (see text for details).[5]

Substance	Compatible Concentration
Salts/Buffers	
ACES, pH 7.8	25mM
Ammonium sulfate	1.5M
Asparagine	1mM
Bicine, pH 8.4	20mM
Bis-Tris, pH 6.5	33mM
Borate (50mM), pH 8.5 (# 28384)	undiluted
B-PER™ Reagent (#78248)	undiluted
Calcium chloride in TBS, pH 7.2	10mM
Na-Carbonate/Na-Bicarbonate (0.2M), pH 9.4 (# 28382)	undiluted
Cesium bicarbonate	100mM
CHES, pH 9.0	100mM
Na-Citrate (0.6M), Na-Carbonate (0.1M), pH 9.0 (# 28388)	1:8 dilution*
Na-Citrate (0.6M), MOPS (0.1M), pH 7.5 (#28386)	1:8 dilution*
Cobalt chloride in TBS, pH 7.2	0.8mM
EPPS, pH 8.0	100mM
Ferric chloride in TBS, pH 7.2	10mM
Glycine-HCl, pH 2.8	100mM
Guanidine-HCl	4M
HEPES, pH 7.5	100mM
Imidazole, pH 7.0	50mM
MES, pH 6.1	100mM
MES (0.1M), NaCl (0.9%), pH 4.7 (#28390)	undiluted
MOPS, pH 7.2	100mM
Modified Dulbecco's PBS, pH 7.4 (#28374)	undiluted
Nickel chloride in TBS, pH 7.2	10mM
PBS: Phosphate (0.1M), NaCl (0.15M), pH 7.2 (# 28372)	undiluted
PIPES, pH 6.8	100mM
RIPA lysis buffer; 50mM Tris, 150mM NaCl, 0.5% DOC, 1% NP-40, 0.1% SDS, pH 8.0	undiluted
Sodium acetate, pH 4.8	200mM
Sodium azide	0.2%
Sodium bicarbonate	100mM
Sodium chloride	1M
Sodium citrate, pH 4.8 or pH 6.4	200mM
Sodium phosphate	100mM
Tricine, pH 8.0	25mM
Triethanolamine, pH 7.8	25mM
Tris	250mM
TBS; Tris (25mM), NaCl (0.15M), pH 7.6 (# 28376)	undiluted
Tris (25mM), Glycine (192mM), pH 8.0 (# 28380)	1:3 dilution*

Substance	Compatible Concentration
Detergents**	
Brij™-35	5.0%
Brij-56, Brij-58	1.0%
CHAPS, CHAPSO	5.0%
Deoxycholic acid	5.0%
Octyl β-glucoside	5.0%
Nonidet P-40 (NP-40)	5.0%
Octyl β-thioglucopyranoside	5.0%
SDS	5.0%
Span™ 20	1.0%
Triton™ X-100	5.0%
Triton X-114, X-305, X-405	1.0%
Tween™-20, Tween-60, Tween-80	5.0%
Zwittergent™ 3-14	1.0%
Chelating agents	
EDTA	10mM
EGTA	——
Sodium citrate	200mM
Reducing & Thiol-Containing Agents	
N-acetylglucosamine in PBS, pH 7.2	10mM
Ascorbic acid	——
Cysteine	——
Dithioerythritol (DTE)	1mM
Dithiothreitol (DTT)	1mM
Glucose	10mM
Melibiose	——
2-Mercaptoethanol	0.01%
Potassium thiocyanate	3.0M
Thimerosal	0.01%
Misc. Reagents & Solvents	
Acetone	10%
Acetonitrile	10%
Aprotinin	10mg/L
DMF, DMSO	10%
DMSO	10%
Ethanol	10%
Glycerol (Fresh)	10%
Hydrazides	——
Hydrides (Na_2BH_4 or $NaCNBH_3$)	——
Hydrochloric Acid	100mM
Leupeptin	10mg/L

* Diluted with ultrapure water.
** Detergents were tested using high-purity Thermo Scientific™ Surfact-Amps™ Products, which have low peroxide content.
-- Dashed-line entry indicates that the material is incompatible with the assay.

[5] For a more extensive list of substances, download Tech Tip # 68: Protein assay compatibility table from our website. This Tech Tip includes compatible substances for all of our protein assays and enables easy comparisons.

Cited References

1. Smith, P.K., *et al.* (1985). Measurement of protein using bicinchoninic acid. *Anal Biochem* **150**:76-85.
2. Wiechelman, K., *et al.* (1988). Investigation of the bicinchoninic acid protein assay: Identification of the groups responsible for color formation. *Anal Biochem* **175**:231-7.
3. Kessler, R. and Fanestil, D. (1986). Interference by lipids in the determination of protein using bicinchoninic acid. *Anal Biochem* **159**:138-42.
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Pronaleck, W.C., *et al.* (2002). Chlamydia trachomatis disrupts N-cadherin-dependent cell-cell junctions and sequester β-catenin in human cervical epithelial cells. *Infection and Immunity* 70:2605-13.

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Figure 104- BCA protein assay instruction manual 1/2

Standards	OD1	OD2	OD Ave	OD - blank		
2000	1.171	1.112	1.1415	0.9420		
1000	0.747	0.735	0.741	0.5415		
500	0.506	0.483	0.4945	0.2950		
250	0.359	0.342	0.3505	0.1510	m	0.0005
125	0.306	0.301	0.3035	0.1040	c	0.0394
0	0.202	0.197	0.1995			

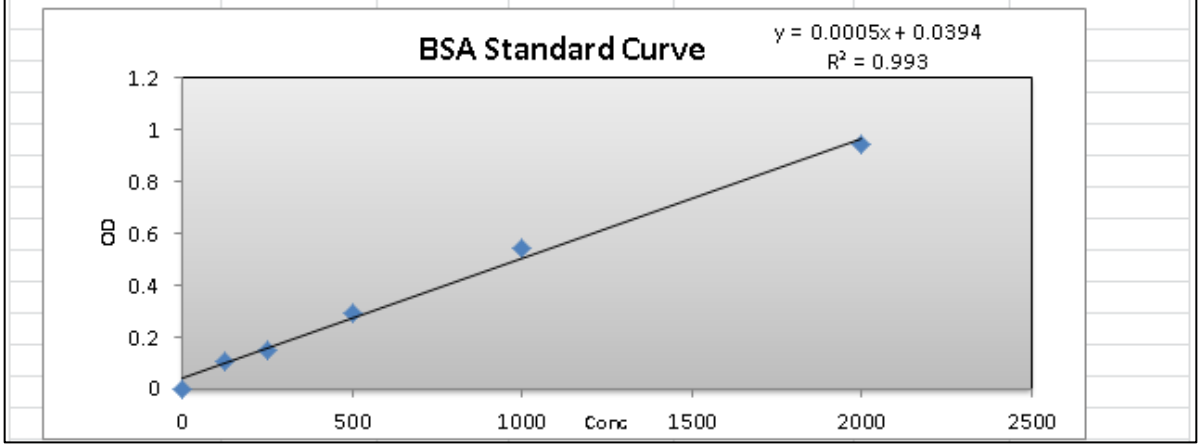

Figure 105- BCA protein assay, HC NE standard curve

Standards	OD1	OD2	OD Ave	OD - blank		
2000	0.988	0.983	0.9855	0.8035		
1000	0.639	0.557	0.598	0.4160		
500	0.382	0.368	0.375	0.1930		
250	0.277	0.264	0.2705	0.0885	**m**	0.0004
125	0.261	0.256	0.2585	0.0765	**c**	0.0041
0	0.183	0.181	0.182			

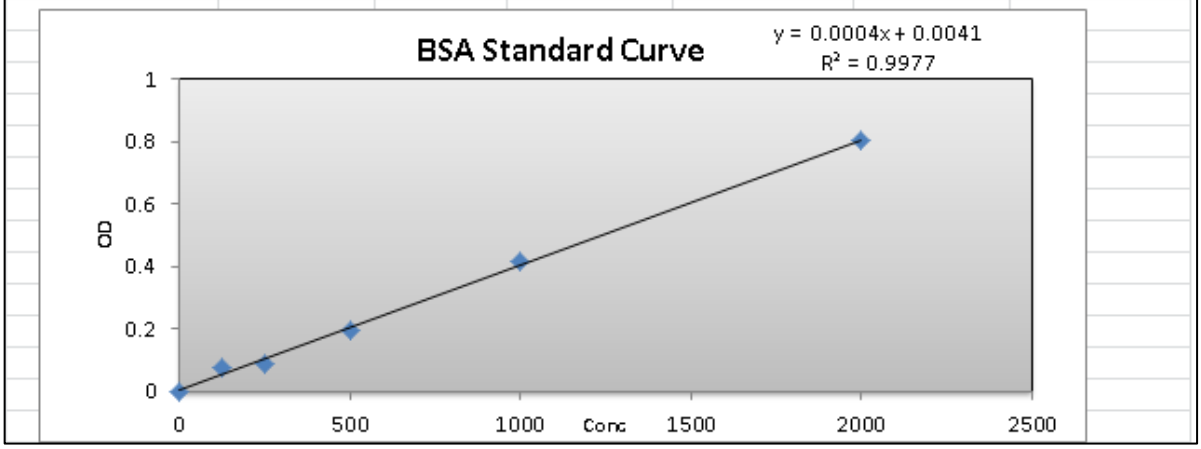

Figure 106- BCA protein assay, PFC NE standard curve

Standards	OD1	OD2	OD Ave	OD - blank		
2000	1.083	1.009	1.046	0.8625		
1000	0.642	0.633	0.6375	0.4540		
500	0.387	0.354	0.3705	0.1870		
250	0.294	0.291	0.2925	0.1090	**m**	0.0004
125	0.274	0.266	0.27	0.0865	**c**	0.0058
0	0.183	0.184	0.1835			

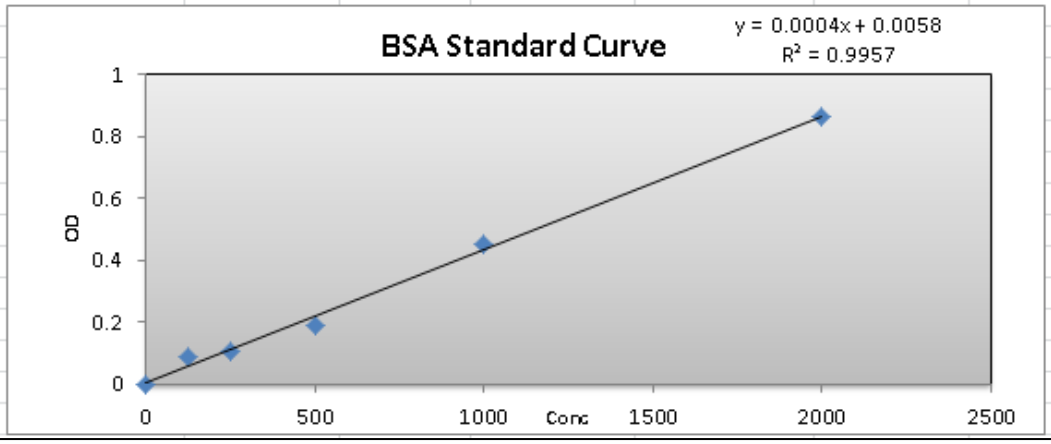

Figure 107- BCA protein assay, HC Glu standard curve

Table 104- ELISA and BCA assay, Data

Rat #	Sex	Treatment	Sex_treatment	ELISA PFC NE ng/ml	BCA PFC NE µg/ml	PFC: NE (pg/g protein)	PFC: NE (ng/g wet weight)	ELISA HC NE ng/ml	BCA HC NE µg/ml	HC: NE (pg/g protein)	HC: NE (ng/g wet weight)	ELISA HC Glu µg/ml	BCA HC Glu µg/ml	HC: Glu (mg/g protein)	HC: Glu (mg/g wet weight)	ELISA PFC Glu µmol/l	ELISA PFC Glu µg/ml	PFC: Glu (mg/g wet weight)
4	F	S	FS	0.6586	166.8	3.948	658.6	0.9790	77.86	12.57	979.0	2.063	102.17	20.19	2.063	14.78	2.173	2.173
8	F	I	FI	0.5552	142.1	3.907	555.2	0.6024	72.43	8.32	602.4	0.956	54.58	17.51	0.956	12.48	1.835	1.835
9	M	S	MS	0.6032	153.3	3.936	603.2	0.8122	89.66	9.06	812.2					12.76	1.877	1.877
10	M	S	MS					0.6097	90.87	6.71		1.336	90.57	14.75	1.336			
11	M	S	MS									1.468	80.03	18.34	1.468			
13	M	I	MI	0.6127	156.9	3.906	612.7	0.5526	84.48	6.54	552.6	1.639	83.80	19.56	1.639	11.02	1.621	1.621
14	M	I	MI					0.5337	74.60	7.15		1.235	83.34	14.82	1.235			
19	F	S	FS	0.3803	141.2	2.693	380.3	0.7588	94.60	8.02	758.8	2.046	97.35	21.02	2.046	9.08	1.335	1.335
20	F	S	FS					0.6472	58.82	11.00		1.920	73.25	26.21	1.920			
23	F	I	FI	0.4591	174.0	2.638	459.1	0.7357	92.07	7.99	735.7	1.699	98.86	17.19	1.699	10.01	1.473	1.473
24	F	I	FI					0.8825	67.86	13.01		1.449	71.90	20.16	1.449			
28	M	S	MS	0.5605	117.7	4.762	560.5	0.5892	92.31	6.38	589.2	1.595	97.80	16.31	1.595	11.65	1.713	1.713
32	M	I	MI	0.6380	173.4	3.679	638.0	0.6407	96.53	6.64	640.7	2.423	121.90	19.88	2.423	22.03	3.240	3.240
35	F	S	FS	0.5428	195.4	2.778	542.8	0.7076	95.33	7.42	707.6	0.642	55.78	11.51	0.642	11.51	1.693	1.693
36	F	S	FS					0.6060	49.42	12.26		1.608	50.06	32.13	1.608			
39	F	I	FI	0.5904	123.7	4.771	590.4	0.6199	79.90	7.76	619.9	1.747	75.96	22.99	1.747	10.13	1.490	1.490
40	F	I	FI					0.6247	70.87	8.82		1.595	77.47	20.59	1.595			
43	M	S	MS	0.6128	159.9	3.833	612.8	0.5646	89.42	6.31	564.6	1.611	77.47	20.79	1.611	11.89	1.748	1.748
44	M	S	MS					0.6232	97.49	6.39		1.874	71.60	26.17	1.874			
47	M	I	MI	0.5803	165.0	3.517	580.3	0.6981	87.98	7.93	698.1	1.746	77.02	22.67	1.746	10.20	1.500	1.500
48	M	I	MI					0.7668	90.14	8.51		1.672	105.48	15.85	1.672			
51	F	S	FS	0.5188	140.0	3.706	518.8	0.6933	71.47	9.70	693.3	1.845	64.22	28.73	1.845	8.75	1.286	1.286
59	M	S	MS	0.7144	133.4	5.356	714.4	0.5816	76.41	7.61	581.6	1.557	50.06	31.11	1.557	9.60	1.412	1.412
60	M	S	MS					0.8512	87.98	9.68		1.896	94.94	19.97	1.896			
63	M	I	MI	0.4747	170.1	2.790	474.7	0.6026	70.27	8.58	602.6	1.777	75.36	23.58	1.777	9.72	1.429	1.429
64	M	I	MI					0.5643	35.69	15.81		1.527	83.64	18.25	1.527			
67	F	S	FS	0.4692	153.9	3.050	469.2	0.8098	71.35	11.35	809.8	2.050	61.96	33.09	2.050	11.38	1.674	1.674
71	F	I	FI	0.5117	191.5	2.672	511.7	0.7217	92.92	7.77	721.7	2.109	115.27	18.30	2.109	8.94	1.315	1.315
72	F	I	FI					0.9609				1.720	78.37	21.95	1.720			
75.1	F	S	FS					0.6960	83.04	8.38		1.610	103.37	15.58	1.610			
76	M	S	MS	0.6004	242.4	2.477	600.4	0.6575	77.61	8.47	657.5	2.079	54.88	37.88	2.079	10.80	1.588	1.588
78.1	F	I	FI	0.6628	208.7	3.176	662.8	0.8342	50.39	16.56	834.2	2.079	78.67	26.42	2.079	12.02	1.767	1.767
80	M	I	MI	0.6110	177.0	3.451	611.0	0.5503	91.47	6.02	550.3	2.424	80.18	30.23	2.424	11.05	1.625	1.625
82.1	M	S	MS	0.6172	143.0	4.316	617.2	0.6768	84.24	8.03	676.8	1.633	89.97	18.15	1.633	12.20	1.794	1.794
83.1	M	S	MS					0.6361	87.49	7.27		1.629	64.67	25.18	1.629			
84	F	S	FS	0.4665	176.1	2.648	466.5	0.7277	69.42	10.48	727.7	1.481	87.56	16.91	1.481	9.49	1.395	1.395
86.1	M	I	MI	0.4822	129.2	3.733	482.2	0.8661	75.45	11.48	866.1	1.595	88.92	17.93	1.595	10.08	1.483	1.483
87	F	I	FI	0.4523	160.2	2.824	452.3	0.6847	98.58	6.95	684.7	1.696	73.86	22.96	1.696	10.35	1.522	1.522
87.1	M	I	MI					0.6080	66.53	9.14		1.553	68.58	22.64	1.553			
98.1	F	S	FS					0.6135	67.61	9.07		1.690	87.41	19.33	1.690			
100.1	F	I	FI					0.6157	76.89	8.01		1.842	44.94	40.98	1.842			

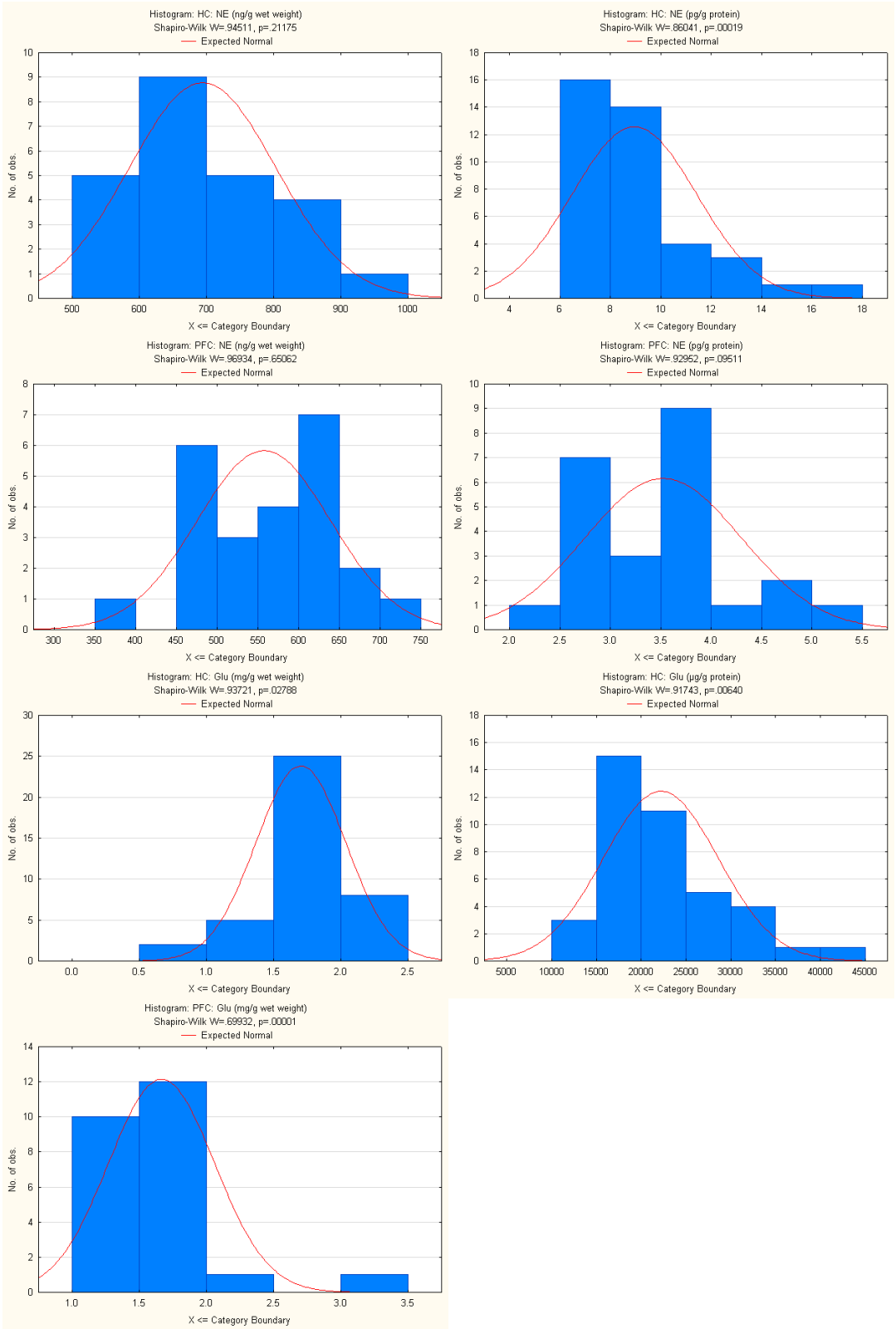

Figure 108- ELISA and BCA assay, Histograms

Table 105- ELISA and BCA assay, Descriptive statistics

Variable	Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
PFC: NE (pg/g protein)	F	S	6	3.1371	2.6484	3.9483	0.5575
PFC: NE (ng/g wet weight)	F	S	6	506.0333	380.3000	658.6000	93.2950
HC: NE (ng/g wet weight)	F	S	6	779.3417	693.3000	979.0000	106.2395
PFC: NE (pg/g protein)	F	I	6	3.3314	2.6380	4.7715	0.84883
PFC: NE (ng/g wet weight)	F	I	6	538.5833	452.3000	662.8000	81.08759
HC: NE (ng/g wet weight)	F	I	6	699.7333	602.4000	834.2000	84.77531
PFC: NE (pg/g protein)	M	S	6	4.1132	2.4768	5.3564	0.97927
PFC: NE (ng/g wet weight)	M	S	6	618.0833	560.5000	714.4000	51.29454
HC: NE (ng/g wet weight)	M	S	6	646.9417	564.5500	812.1500	92.40190
PFC: NE (pg/g protein)	M	I	6	3.5127	2.7904	3.9058	0.3889
PFC: NE (ng/g wet weight)	M	I	6	566.4833	474.7000	638.0000	70.6415
HC: NE (ng/g wet weight)	M	I	6	651.7000	550.2500	866.1000	118.9750

Variable	Sex_treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
HC: NE (pg/g protein)	FS	10	10.09109	7.42300	12.57459	8.38189	11.34908
HC: Glu (mg/g protein)	FS	10	20.60711	11.50617	33.09105	16.91407	28.73311
HC: Glu (mg/g wet weight)	FS	10	1.76735	0.64185	2.06320	1.60830	2.04630
PFC: Glu (mg/g wet weight)	FS	6	1.53478	1.28630	2.17290	1.33530	1.69335
HC: NE (pg/g protein)	FI	9	8.00673	6.94524	16.55634	7.76672	8.81505
HC: Glu (mg/g protein)	FI	10	21.26709	17.19076	40.97819	18.29600	22.99251
HC: Glu (mg/g wet weight)	FI	10	1.70978	0.95560	2.10900	1.59480	1.84155
PFC: Glu (mg/g wet weight)	FI	6	1.50603	1.31540	1.83465	1.47250	1.76735
HC: NE (pg/g protein)	MS	10	7.44062	6.31335	9.67538	6.39219	8.47072
HC: Glu (mg/g protein)	MS	10	20.37979	14.75451	37.87843	18.15052	26.17101
HC: Glu (mg/g wet weight)	MS	10	1.61975	1.33635	2.07875	1.55745	1.87375
PFC: Glu (mg/g wet weight)	MS	6	1.73073	1.41190	1.87705	1.58795	1.79370
HC: NE (pg/g protein)	MI	10	8.22017	6.01564	15.81119	6.63731	9.13872
HC: Glu (mg/g protein)	MI	10	19.71817	14.81941	30.22921	17.93385	22.67325
HC: Glu (mg/g wet weight)	MI	10	1.65530	1.23510	2.42380	1.55295	1.77735
PFC: Glu (mg/g wet weight)	MI	6	1.56035	1.42940	3.23985	1.48275	1.62460

Table 106- ELISA, HC NE (ng/g wet weight) statistics

Parametric factorial ANOVA for all sex and housing groups. Followed by Bonferroni post hoc.

	Univariate Tests of Significance for HC: NE (ng/g wet weight) (5 ELISA BCA) Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	11573565	1	11573565	1124.552	0.000000
Sex	48834	1	48834	4.745	0.041521
Treatment	8404	1	8404	0.817	0.376944
Sex*Treatment	10677	1	10677	1.037	0.320591
Error	205834	20	10292		

	Bonferroni test; variable HC: NE (ng/g wet weight) (5 ELISA BCA) Probabilities for Post Hoc Tests Error: Between MS = 10292., df = 20.000		
Cell No.	Sex	{1} 739.54	{2} 649.32
1	F		0.041521
2	M	0.041521	

Table 107- ELISA and BCA assay, HC NE (pg/g protein) statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); HC: NE (pg/g protein) (5 ELISA BCA) Independent (grouping) variable: Sex_treatment Kruskal-Wallis test: H (3, N= 39) =7.854615 p =.0491			
Depend.: HC: NE (pg/g protein)	FS R:27.400	FI R:21.000	MS R:13.400	MI R:18.300
FS		1.000000	0.036237	0.445902
FI	1.000000		0.881132	1.000000
MS	0.036237	0.881132		1.000000
MI	0.445902	1.000000	1.000000	

Table 108- ELISA, PFC NE (ng/g wet weight) statistics

Parametric factorial ANOVA for all sex and housing groups. Followed by Bonferroni post hoc.

	Univariate Tests of Significance for PFC: NE (ng/g wet weight) (5 ELISA BCA) Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	7453888	1	7453888	1301.960	0.000000
Sex	29379	1	29379	5.132	0.034752
Treatment	544	1	544	0.095	0.761003
Sex*Treatment	10622	1	10622	1.855	0.188313
Error	114503	20	5725		

	Bonferroni test; variable PFC: NE (ng/g wet weight) (5 ELISA BCA) Probabilities for Post Hoc Tests Error: Between MS = 5725.1, df = 20.000		
Cell No.	Sex	{1} 522.31	{2} 592.28
1	F		0.034752
2	M	0.034752	

Table 109- ELISA and BCA assay, PFC NE (pg/g protein) statistics

Parametric factorial ANOVA for all sex and housing groups.

	Univariate Tests of Significance for PFC: NE (pg/g protein) (5 ELISA BCA) Sigma-restricted parameterization Effective hypothesis decomposition				
Effect	SS	Degr. of Freedom	MS	F	p
Intercept	297.9810	1	297.9810	556.5642	0.000000
Sex	2.0095	1	2.0095	3.7534	0.066953
Treatment	0.2476	1	0.2476	0.4625	0.504268
Sex*Treatment	0.9474	1	0.9474	1.7696	0.198399
Error	10.7079	20	0.5354		

Table 110- ELISA, HC Glu (mg/g wet weight) statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); HC: Glu (mg/g wet weight) (5 ELISA BCA) Independent (grouping) variable: Sex_treatment Kruskal-Wallis test: H (3, N= 40) =.7258537 p =.8671			
Depend.: HC: Glu (mg/g wet weight)	FS R:22.100	FI R:21.700	MS R:18.100	MI R:20.100
FS		1.000000	1.000000	1.000000
FI	1.000000		1.000000	1.000000
MS	1.000000	1.000000		1.000000
MI	1.000000	1.000000	1.000000	

Table 111- ELISA and BCA assay, HC Glu (mg/g protein) statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); HC: Glu (mg/g protein) (5 BCA ELISA) Independent (grouping) variable: Sex_treatment Kruskal-Wallis test: H (3, N= 40) =.7829268 p =.8535			
Depend.: HC: Glu (mg/g protein)	FS R:21.100	FI R:22.200	MS R:20.900	MI R:17.800
FS		1.000000	1.000000	1.000000
FI	1.000000		1.000000	1.000000
MS	1.000000	1.000000		1.000000
MI	1.000000	1.000000	1.000000	

Table 112- ELISA, PFC Glu (mg/g wet weight) statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); PFC: Glu (mg/g wet weight) (5 ELISA BCA) Independent (grouping) variable: Sex_treatment Kruskal-Wallis test: H (3, N= 24) =1.886667 p =.5963			
Depend.: PFC: Glu (mg/g wet weight)	FS R:10.333	FI R:11.500	MS R:15.667	MI R:12.500
FS		1.000000	1.000000	1.000000
FI	1.000000		1.000000	1.000000
MS	1.000000	1.000000		1.000000
MI	1.000000	1.000000	1.000000	

A.5 ULTRASONIC VOCALISATIONS

Table 113- USV, Data

Rat #	Channel	Sex	Treatment	Sex_ Treatment	Mean Call Duration (ms) Raw	Total Call Number Raw	MCD (ms) (Channel A corrected ÷1.42)	TCN (Channel A corrected ÷4.58)
115	A	F	S	FS	675.57	217	475.75	47
121	A	F	S	FS	523.83	157	368.90	34
123	A	F	S	FS	757.46	244	533.42	53
129	A	F	S	FS	532.66	94	375.11	21
130	A	F	S	FS	596.48	196	420.06	43
144	A	F	S	FS	536.09	163	377.53	36
118	A	F	I	FI	601.75	219	423.77	48
119	A	F	I	FI	568.53	146	400.37	32
126	A	F	I	FI	569.69	216	401.19	47
128	A	F	I	FI	617.29	224	434.71	49
135	A	F	I	FI	625.68	204	440.62	45
136	A	F	I	FI	513.86	118	361.88	26
146	A	F	I	FI	597.98	249	421.11	54
148	A	F	I	FI	563.56	182	396.87	40
137	A	M	S	MS	667.46	210	470.04	46
139	A	M	S	MS	486.65	130	342.71	28
141	A	M	I	MI	603.44	174	424.96	38
113	B	F	S	FS	417.76	45	417.76	45
114	B	F	S	FS	419.25	52	419.25	52
122	B	F	S	FS	398.47	15	398.47	15
124	B	F	S	FS	431.00	31	431.00	31
131	B	F	S	FS	421.36	33	421.36	33
132	B	F	S	FS	357.50	2	357.50	2
143	B	F	S	FS	512.29	17	512.29	17
145	B	F	S	FS	429.37	51	429.37	51
120	B	F	I	FI	452.28	69	452.28	69
125	B	F	I	FI	466.47	19	466.47	19
127	B	F	I	FI	454.42	55	454.42	55
133	B	F	I	FI	426.66	44	426.66	44
134	B	F	I	FI	362.38	8	362.38	8
147	B	F	I	FI	440.28	43	440.28	43
116	B	F	I	FI	415.75	8	415.75	8
117	B	F	I	FI	384.77	13	384.77	13
138	B	M	S	MS	380.63	24	380.63	24
101	B	M	S	MS	427.81	73	427.81	73
102	B	M	S	MS	366.47	17	366.47	17
140	B	M	I	MI	388.81	26	388.81	26
142	B	M	I	MI	416.50	4	416.50	4
108	B	M	I	MI	410.46	56	410.46	56
109	B	M	I	MI	459.87	116	459.87	116
110	B	M	I	MI	464.88	98	464.88	98

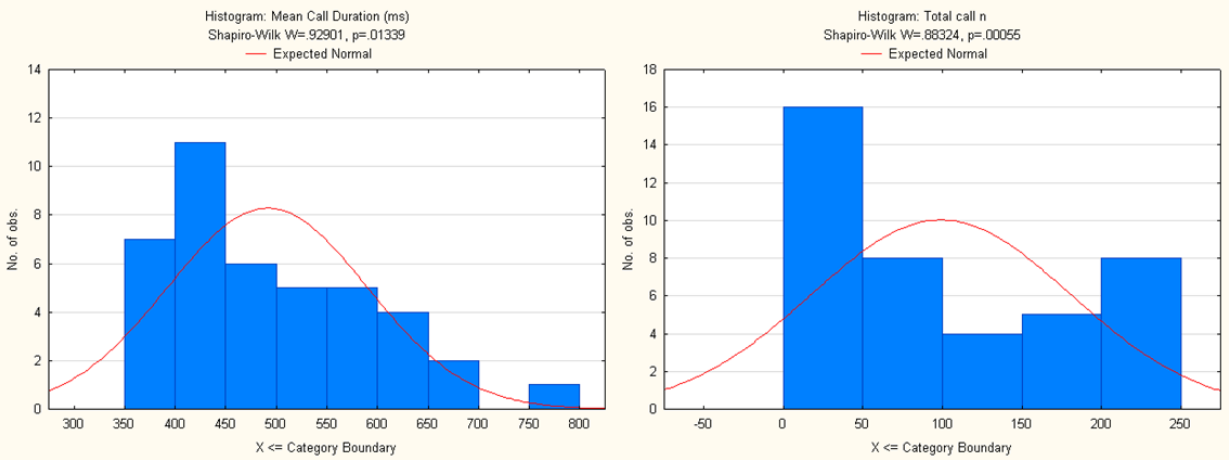

Figure 109- USV, Histograms, raw data

Table 114- USV, Descriptive statistics, raw data

Variable	Sex_Treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
Mean Call Duration (ms)	FS	14	471.6471	357.5000	757.4590	419.2500	536.0920
Total call n	FS	14	51.5000	2.0000	244.0000	31.0000	163.0000
Mean Call Duration (ms)	FI	16	490.1690	362.3750	625.6765	433.4691	583.8305
Total call n	FI	16	93.5000	8.0000	249.0000	31.0000	210.0000
Mean Call Duration (ms)	MS	5	427.8082	366.4706	667.4571	380.6250	486.6462
Total call n	MS	5	73.0000	17.0000	210.0000	24.0000	130.0000
Mean Call Duration (ms)	MI	6	438.1853	388.8077	603.4425	410.4643	464.8776
Total call n	MI	6	77.0000	4.0000	174.0000	26.0000	116.0000

Variable	Channel	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
Mean Call Duration (ms)	A	17	596.4796	486.6462	757.4590	536.0920	617.2902
Total call n	A	17	196.0000	94.0000	249.0000	157.0000	217.0000
Mean Call Duration (ms)	B	24	420.3068	357.5000	512.2941	393.6372	446.2772
Total call n	B	24	32.0000	2.0000	116.0000	16.0000	53.5000

Table 115- USV, Mean call duration (ms) statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); Mean Call Duration (ms) (USV raw) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 41) =1.786067 p =.6180			
Depend.: Mean Call Duration (ms)	FS R:21.429	FI R:23.313	MS R:17.000	MI R:17.167
FS		1.000000	1.000000	1.000000
FI	1.000000		1.000000	1.000000
MS	1.000000	1.000000		1.000000
MI	1.000000	1.000000	1.000000	

Table 116- USV, Total call number statistics

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups

	Multiple Comparisons p values (2-tailed); Total call n (USV raw) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 41) =.6207569 p =.8917			
Depend.: Total call n	FS R:20.179	FI R:22.750	MS R:20.300	MI R:18.833
FS		1.000000	1.000000	1.000000
FI	1.000000		1.000000	1.000000
MS	1.000000	1.000000		1.000000
MI	1.000000	1.000000	1.000000	

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When nonparametric Kruskal Wallis test was applied to raw data sets neither housing nor sex differences were found between groups. It was noted that the Channel A equipment seemed to be recording with a greater sensitivity than the Channel B equipment. This was confirmed with a Mann Whitney U test. A difference was found ($H_{(3,\ N=175)}$ = 15.45, p= 0.0015) where channel A was greater than channel B for both mean call duration and total call number values ($p<0.0001$). This was thought to be due to the age of the batteries in the different bat detectors. Channel A recordings were found to be greater than in Channel B by a factor of 1.42 for mean call duration recordings and a factor of 4.58 for total call number. These factors were therefore applied as a correction so that the two channels were comparable. Since socialised and isolated animals were swapped between Channel A and B detectors for each test, no bias was created and therefore a correction of this nature was appropriate.

Table 117- USV, Channel A vs. Channel B statistics

Comparing two independent sample groups, Mann Whitney U test.

	Mann-Whitney U Test (w/ continuity correction) (USV raw) By variable Channel Marked tests are significant at p <.05000									
variable	Rank Sum A	Rank Sum B	U	Z	p-value	Z adjusted	p-value	Valid N A	Valid N B	2*1sided exact p
Mean Call Duration (ms)	560.0000	301.0000	1.000000	5.358718	0.000000	5.358718	0.000000	17	24	0.000000
Total call n	559.0000	302.0000	2.000000	5.332255	0.000000	5.332720	0.000000	17	24	0.000000

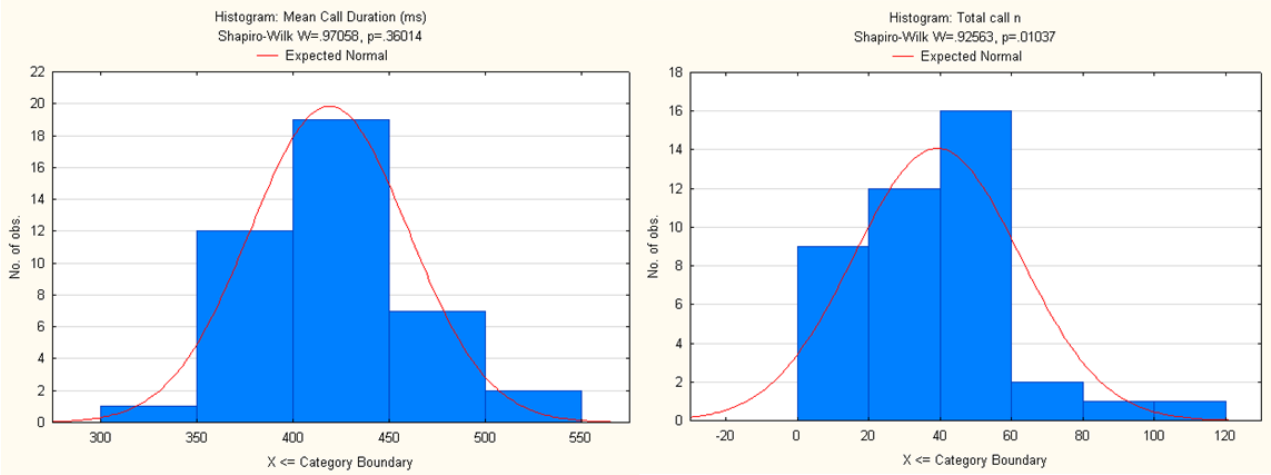

Figure 110- USV, Histograms, corrected data

Table 118- USV, Descriptive statistics, corrected data

Variable	Aggregate Results Descriptive Statistics (USV corrected) Sex	Treatment	Valid N	Mean	Minimum	Maximum	Std.Dev.
Mean Call Duration (ms)	F	S	14	424.1266	357.5000	533.4218	52.08652
Mean Call Duration (ms)	F	I	16	417.7195	361.8763	466.4737	31.24923
Mean Call Duration (ms)	M	S	5	397.5305	342.7086	470.0402	51.06457
Mean Call Duration (ms)	M	I	6	427.5800	388.8077	464.8776	29.52852

Variable	Aggregate Results Descriptive Statistics (USV corrected) Sex_Treatment	Valid N	Median	Minimum	Maximum	Lower Quartile	Upper Quartile
Total call n	FS	14	34.93450	2.000000	53.27511	20.52402	47.37991
Total call n	FI	16	43.50000	8.000000	69.00000	22.38210	48.36245
Total call n	MS	5	28.38428	17.00000	73.00000	24.00000	45.85153
Total call n	MI	6	46.99563	4.000000	116.0000	26.00000	98.00000

Table 119- USV, Mean call duration (ms) statistics, corrected data

Parametric factorial ANOVA for all sex and housing groups.

Effect	Univariate Tests of Significance for Mean Call Duration (ms) (USV corrected) Sigma-restricted parameterization Effective hypothesis decomposition SS	Degr. of Freedom	MS	F	p
Intercept	5550881	1	5550881	3174.049	0.000000
Sex	559	1	559	0.320	0.575066
Treatment	1117	1	1117	0.638	0.429363
Sex*Treatment	2655	1	2655	1.518	0.225672
Error	64707	37	1749		

Table 120- USV, Total call number statistics , corrected data

Nonparametric test comparing multiple independent sample groups, Kruskal Wallis test for all sex_housing groups.

	Multiple Comparisons p values (2-tailed); Total call n (USV corrected) Independent (grouping) variable: Sex_Treatment Kruskal-Wallis test: H (3, N= 41) =1.211535 p =.7502			
Depend.: Total call n	FS R:19.250	FI R:21.250	MS R:19.700	MI R:25.500
FS		1.000000	1.000000	1.000000
FI	1.000000		1.000000	1.000000
MS	1.000000	1.000000		1.000000
MI	1.000000	1.000000	1.000000	